This Week in The Journal

● Cellular/Molecular

Glycine Receptor Activation: When Three Out of Five Is Enough

Marco Beato, Paul J. Groot-Kormelink, David Colquhoun, and Lucia G. Sivilotti
(see pages 895–906)

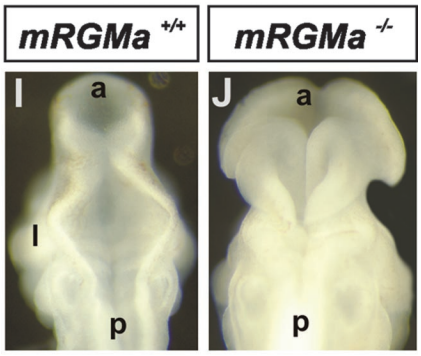
The prototypic member of the acetylcholine receptor family, the muscle nicotinic receptor, is a heteromeric complex with five subunits but only two ligand-binding sites. However, some members of this receptor superfamily are homomeric, containing five seemingly identical binding sites. Whether activation actually requires binding of five agonist molecules in this situation is unclear. Such questions cannot be answered using classical pharmacological methods. Thus Beato et al. analyzed the single-channel activity of recombinant glycine receptors containing five $\alpha 1$ subunits, the principal juvenile form of this inhibitory synaptic receptor. The channels opened more efficaciously as the glycine concentration increased, but gating saturated when three glycine molecules were bound. They could not resolve whether the fourth and fifth bindings occur or are silent. The three out of five odds may be a general rule, because similar results have been suggested for homomeric $GABA_C$ and 5-HT3 channels.

▲ Development/Plasticity/Repair

The Mouse Repulsive Guidance Molecule (RGM) Family

Vera Niederkofler, Rishard Salie, Markus Sigrist, and Silvia Arber
(see pages 808 – 818)

Topographically organized axonal projections are guided by gradients of attractive and repulsive molecules, an idea first espoused by Roger Sperry to explain the pattern of retinal ganglia cell termination zones in the chick optic tectum. The search for molecules expressed in such patterns led to the Ephrins and the less-characterized chick repulsive guidance molecule (cRGM). In an effort to define an *in vivo* role for RGM, Niederkofler et al. have now identified three mouse homologs. Mouse RGMa (mRGMa) and mRGMb were expressed in a nonoverlapping pattern in the nervous system, whereas mRGMc was confined to skeletal muscle. Despite its expression in the superior colliculus, mRGMa is not essential for patterning of ganglion termination zones, as demonstrated by normal retinotectal mapping in mRGMa-deficient mice. Instead, one-half of the mRGMa-deficient embryos were exencephalic (they failed to close the neural tube), suggesting a more significant role in early development. The latter function appears to be a role shared with the Ephrins.



Dorsal head view of embryonic day 10.5 mice showing exencephalic phenotype in mRGMa-deficient mice (*J*) compared with wild-type mice (*I*).

■ Behavioral/Systems/Cognitive

Painful Memories

Thomas Klein, Walter Magerl, Hanns-Christian Hopf, Jürgen Sandkühler, and Rolf-Detlef Treede
(see pages 938 –946)

Long-term potentiation (LTP) and long-term depression (LTD) are among the best-studied examples of synaptic plasticity. These lasting changes in synaptic efficacy are candidate mechanisms for learning and memory, but there is little definitive evidence linking these cellular mechanisms to human behavior. In this week's *Journal*, Klein et al. build on observations that high- or low-frequency electrical stimulation induces LTP- and LTD-like phenomena in spinal nociceptive pathways in animals. They focused on a behavioral correlate that is easy to evoke and measure in humans: pain. While both stimulation patterns led to a corresponding change in perception (hyperalgesia with LTP-like stimuli and hypoalgesia with LTD-like stimuli), the specific outcomes and mechanisms differed subtly. Although the underlying mechanisms may be complex, these results add to the idea that LTP-like plasticity contributes to hyperalgesia and chronic pain, whereas LTD-like plasticity may contribute to the analgesia associated with treatments such as transcutaneous electric nerve stimulation (TENS) and perhaps its ancient relative, acupuncture.

♦ Neurobiology of Disease

CRE, Transcription, and HD

Karl Obrietan and Kari R. Hoyt
(see pages 791–796)

The neurodegeneration in Huntington's disease (HD) has been linked to polyglutamine repeats in the huntingtin protein. The consequences of this mutation, and indeed the normal function of the protein, remain a mystery. Recently, *in vitro* evidence suggested that huntingtin-related intranuclear inclusions interfere with cAMP-response element (CRE)-mediated gene transcription, presumably by binding of mutant huntingtin with CREB-binding protein (CBP). However, in this issue Obrietan and Hoyt report facilitated gene transcription in an HD animal *in vivo*. They crossed an HD mouse with a mouse that expressed a CRE-dependent marker protein. In addition to increased CRE-mediated marker expression (notably in the striatum), the HD mice also expressed elevated amounts of phosphorylated CREB and a CREB-regulated protein. Intracellular inclusions were present in the HD mice; thus they were not sufficient to prevent transcription or translation. Why the discrepancy with previous reports? The authors wonder if high expression levels of huntingtin as used in the previous *in vitro* studies may allow a low-affinity interaction between CBP and huntingtin.

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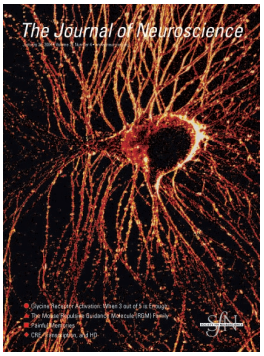

Cover picture: Second-harmonic generation microscopy image of a primary cultured *Aplysia* neuron stained with the membrane dye DHPESBP. The signal is modulated by membrane potential and was found to be capable of recording action potentials with 0.6 μm and 0.833 msec spatiotemporal resolution. The high-resolution and deep tissue-imaging capability of this nonlinear microscopy technique should prove valuable to future electrophysiology studies. For details, see the article by Dombeck et al. in this issue (pages 999–1003).

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Brief Communications

CRE-Mediated Transcription Is Increased in Huntington's Disease Transgenic Mice

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Disruption of cAMP response element (CRE)-dependent transcription has been hypothesized to contribute to neuronal death and dysfunction in Huntington's disease (HD) and other polyglutamine repeat disorders. Whether dysregulation of CRE-dependent transcription actually occurs *in vivo* in response to expression of expanded polyglutamine repeats has not been tested. We directly tested whether CRE-dependent transcription is affected *in vivo* by cross breeding a transgenic mouse model of HD (line R6/2) with a transgenic mouse that expresses a CRE-regulated reporter gene. Instead of compromised CRE-dependent transcription in HD mice, we found a robust upregulation of CRE-dependent transcription in several brain regions (striatum, hippocampus, cortex). CRE-mediated transcription was also evoked by striatal forskolin infusion and by photic stimulation in HD animals. Increased cAMP response element-binding protein (CREB) phosphorylation and elevated levels of the CREB-regulated gene product, CCAATAQ: A/enhancer binding protein β, were also found in HD mice. Significant alterations in CREB binding protein expression and localization were not observed in symptomatic R6/2 mice. Thus, rather than repressing CRE-mediated transcription, mutant huntingtin appears to facilitate transcription via a CRE-dependent mechanism *in vivo*.

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Optical Recording of Action Potentials with Second-Harmonic Generation Microscopy

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Nonlinear microscopy has proven to be essential for neuroscience investigations of thick tissue preparations. However, the optical recording of fast (~1 msec) cellular electrical activity has never until now been successfully combined with this imaging modality. Through the use of second-harmonic generation microscopy of primary *Aplysia* neurons in culture labeled with 4-[4-(dihexylamino)phenyl][ethynyl]-1-(4-sulfobutyl)pyridinium (inner salt), we optically recorded action potentials with 0.833 msec temporal and 0.6 μm spatial resolution on soma and neurite membranes. Second-harmonic generation response as a function of change in membrane potential was found to be linear with a signal change of ~6%/100 mV. The signal-to-noise ratioAQ: C was ~1 for single-trace action potential recordings but was readily increased to ~6–7 with temporal averaging of ~50 scans. Photodamage was determined to be negligible by observing action potential characteristics, cellular resting potential, and gross cellular morphology during and after laser illumination. High-resolution (micrometer scale) optical recording of membrane potential activity by previous techniques has been limited to imaging depths an order of magnitude less than nonlinear methods.AQ: D Because second-harmonic generation is capable of imaging up to ~400 μm deep into intact tissue with submicron resolution and little out-of-focus photodamage or bleaching, its ability to record fast electrical activity should prove valuable to future electrophysiology studies.

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Articles

CELLULAR/MOLECULAR

Inhibition of N-Type Voltage-Activated Calcium Channels in Rat Dorsal Root Ganglion Neurons by P2Y Receptors Is a Possible Mechanism of ADP-Induced Analgesia

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Patch-clamp recordings from small-diameter rat dorsal root ganglion (DRG) neurons maintained in culture demonstrated preferential inhibition by ATP of high-voltage-activated, but not low-voltage-activated, Ca^{2+} currents (I_{Ca}). The rank order of agonist potency was UTP > ADP > ATP. ATP depressed the ω-conotoxin GVIAAQ: A-sensitive N-type current only. Pyridoxal-5-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) and 2′-deoxy-N^6-methyladenosine 3′,5′-bisphosphate tetraammonium, two $P2Y_1$ receptor antagonists, almost abolished the ATP-induced inhibition. Both patch-clamp recordings and immunocytochemistry coupled with confocal laser microscopy indicated a colocalization of functional $P2X_3$ and $P2Y_1$ receptors on the same DRG neurons. Because the effect of ATP was inhibited by intracellular guanosine 5′-O-(2-thiodiphosphate) or by applying a strongly depolarizing prepulse, $P2Y_1$ receptors appear to block I_{Ca} by a pathway involving the $\beta\gamma$ subunit of a $G_{q/11}$ protein. Less efficient buffering of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) by reducing the intrapipette EGTA failed to interfere with the ATP effect. Fura-2 microfluorimetry

suggested that ATP raised $[Ca^{2+}]_i$ by a Gα-mediated release from intracellular pools and simultaneously depressed the high external potassium concentration-induced increase of $[Ca^{2+}]_i$ by inhibiting I_{Ca} via G$\beta\gamma$. Adenosine 5′-O-(2-thiodiphosphate) inhibited dorsal root-evoked polysynaptic population EPSPs in the hemisected rat spinal cord and prolonged the nociceptive threshold on intrathecal application in the tail-flick assay. These effects were not antagonized by PPADS. Hence, P2Y receptor activation by ADP, which is generated by enzymatic degradation of ATP, may decrease the release of glutamate from DRG terminals in the spinal cord and thereby partly counterbalance the algogenic effect of ATP.

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Morphological and Physiological Features of a Set of Spinal Substantia Gelatinosa Neurons Defined by Green Fluorescent Protein Expression

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The spinal substantia gelatinosa (SG) is known to be involved in the manipulation of nociceptive and thermal primary afferent input; however, the interrelationships of its neuronal components are poorly understood. As a step toward expanding understanding, we took a relatively unique approach by concentrating on a set of SG neurons selectively labeled by green fluorescent protein (GFP) in a transgenic mouse. These GFP-expressing SG neurons prove to have homogenous morphological and electrophysiological properties, are systematically spaced in the SG, contain GABA, receive C-fiber primary afferent input, and upregulate c-Fos protein in response to noxious stimuli. Together, the properties established for these GFP-labeled neurons are consistent with a modular SG organization in which afferent activity related to nociception or other C-fiber signaling are subject to integration/modulation by repeating, similar circuits of neurons.

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Cyclin-Dependent Kinase 5 Phosphorylates the N-Terminal Domain of the Postsynaptic Density Protein PSD-95 in Neurons

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PSD-95 (postsynaptic density 95)AQ: A is a postsynaptic scaffolding protein that links NMDA receptors to the cytoskeleton and signaling molecules. The N-terminal domain of PSD-95 is involved in the synaptic targeting and clustering of PSD-95 and in the clustering of NMDA receptors at synapses. The N-terminal domain of PSD-95 contains three consensus phosphorylation sites for cyclin-dependent kinase 5 (cdk5), a proline-directed serine-threonine kinase essential for brain development and implicated in synaptic plasticity, dopamine signaling, cocaine addiction, and neurodegenerative disorders.

We report that PSD-95 is phosphorylated in the N-terminal domain by cdk5 *in vitro* and *in vivo*, and that this phosphorylation is not detectable in brain lysates of cdk5−/− mice. N-terminal phosphorylated PSD-95 is found in PSD fractions together with cdk5 and its activator, p35, suggesting a role for phosphorylated PSD-95 at synapses. In heterologous cells, coexpression of active cdk5 reduces the ability of PSD-95 to multimerize and to cluster neuronal ion channels, two functions attributed to the N-terminal domain of PSD-95. Consistent with these observations, the lack of cdk5 activity in cultured neurons results in larger clusters of PSD-95. In cdk5−/− cortical neurons, more prominent PSD-95 immunostained clusters are observed than in wild-type neurons. In hippocampal neurons, the expression of DNcdk5 (inactive form of cdk5) or of the triple alanine mutant (T19A, S25A, S35A) full-length PSD-95 results in increased PSD-95 cluster size.

These results identify cdk5-dependent phosphorylation of the N-terminal domain of PSD-95 as a novel mechanism for regulating the clustering of PSD-95. Moreover, these observations support the possibility that cdk5-dependent phosphorylation of PSD-95 dynamically regulates the clustering of PSD-95/NMDA receptors at synapses, thus providing a possible mechanism for rapid changes in density and/or number of receptor at synapses.

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Action Potential Timing Determines Dendritic Calcium during Striatal Up-States

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Up-states represent a key feature of synaptic integration in cortex and striatum that involves activation of many synaptic inputs. In the striatum, the sparse firing and tight control of action potential timing is in contrast to the large intracellular membrane potential depolarizations observed during the up-state. One hallmark of striatal spiny projection neurons is the delay to action potential generation in both up-states and suprathreshold depolarization by somatic current injection. By studying somatic and dendritic intracellular calcium ($[Ca^{2+}]_i$) transients during spontaneous up-states in cortex–AQ: Bstriatum–substantia nigra organotypic cultures, we show that the delay between up-state onset and action potential generation determines dendritic peak $[Ca^{2+}]_i$. Peak $[Ca^{2+}]_i$ from single action potentials reached maximum values when action potentials were close to up-state onset and sharply decayed to near subthreshold up-state $[Ca^{2+}]$ levels as a function of time ($\tau = 47 \pm 26$ msec for tertiary dendrite). Similarly, a precisely timed action potential elicited during subthreshold up-states through somatic current injection established that the delay between up-state onset and action potential generation is the critical variable that controls peak $[Ca^{2+}]_i$. Blocking NMDA channels internally with high intracellular Mg^{2+} ($[Mg^{2+}]_i$) (10 mM) abolished the dependency of peak $[Ca^{2+}]_i$ on action potential timing during spontaneous up-states. Finally, high $[Mg^{2+}]_i$ specifically blocked $[Ca^{2+}]_i$ transients that

resulted from local NMDA application in conjunction with backpropagating action potentials. We conclude that precisely timed, single action potentials during striatal up-states control peak dendritic calcium levels. We suggest that this mechanism might play an important role in synaptic plasticity of the corticostriatal pathway.
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Molecular Basis of Gap Junctional Communication in the CNS of the Leech *Hirudo medicinalis*

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Gap junctions are intercellular channels that allow the passage of ions and small molecules between cells. In the nervous system, gap junctions mediate electrical coupling between neurons. Despite sharing a common topology and similar physiology, two unrelated gap junction protein families exist in the animal kingdom. Vertebrate gap junctions are formed by members of the connexin family, whereas invertebrate gap junctions are composed of innexin proteins. Here we report the cloning of two innexins from the leech *Hirudo medicinalis*. These innexins show a differential expression in the leech CNS: *Hm-inx1* is expressed by every neuron in the CNS but not in glia, whereas *Hm-inx2* is expressed in glia but not neurons. Heterologous expression in the paired *Xenopus* oocyte system demonstrated that both innexins are able to form functional homotypic gap junctions. *Hm-inx1* forms channels that are not strongly gated. In contrast, *Hm-inx2* forms channels that are highly voltage-dependent; these channels demonstrate properties resembling those of a double rectifier. In addition, *Hm-inx1* and *Hm-inx2* are able to cooperate to form heterotypic gap junctions in *Xenopus* oocytes. The behavior of these channels is primarily that predicted from the properties of the constituent hemichannels but also demonstrates evidence of an interaction between the two.

This work represents the first demonstration of a functional gap junction protein from a Lophotrochozoan animal and supports the hypothesis that connexin-based communication is restricted to the deuterostome clade.
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The Activation Mechanism of α1 Homomeric Glycine Receptors

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The glycine receptor mediates fast synaptic inhibition in the spinal cord and brainstem. Its activation mechanism is not known, despite the physiological importance of this receptor and the fact that it can serve as a prototype for other homopentameric channels. We analyzed single-channel recordings from rat recombinant α1 glycine receptors by fitting different mechanisms simultaneously to sets of sequences of openings at four glycine concentrations (10–1000 μM). The adequacy of the mechanism and the rate constants thus fitted was judged by examining how well these described the observed dwell-time distributions, open–shut correlation, and single-channel P_{open} dose–response curve. We found that gating efficacy increased as more glycine molecules bind to the channel, but maximum efficacy was reached when only three (of five) potential binding sites are occupied. Successive binding steps are not identical, implying that binding sites can interact while the channel is shut. These interactions can be interpreted in the light of the topology of the binding sites within a homopentamer.
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Production of 5α-Reduced Neurosteroids Is Developmentally Regulated and Shapes $GABA_A$ Miniature IPSCs in Lamina II of the Spinal Cord

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In lamina II of the spinal dorsal horn, synaptic inhibition mediated by ionotropic $GABA_A$ and glycine receptors contributes to the integration of peripheral nociceptive messages. Whole-cell patch-clamp recordings were performed from lamina II neurons in spinal cord slices to study the properties of miniature IPSCs (mIPSCs)AQ: B mediated by activation of $GABA_A$ and glycine receptors in immature ($<$30 d) and adult rats. Blockade of neurosteroidogenesis by 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide (PK11195),AQ: C an inhibitor of the peripheral benzodiazepine receptor (PBR), or finasteride, which blocks 5α-reductase, accelerated the decay kinetics of $GABA_A$ receptor-mediated mIPSCs in immature, but not in adult animals. Glycine receptor-mediated mIPSCs remained unaffected under these conditions. These results suggest the presence of a tonic production of 5α-reduced neurosteroids in young rats that confers slow decay kinetics to $GABA_A$ mIPSCs. At all of the ages, selective stimulation of PBR by diazepam in the presence of flumazenil prolonged $GABA_A$ mIPSCs in a PK11195- and finasteride-sensitive manner. This condition also increased the proportion of mixed $GABA_A$/glycine mIPSCs in the immature animals and led to the reappearance of mixed $GABA_A$/glycine mIPSCs in the adult. Our results might point to an original mechanism by which the strength of synaptic inhibition can be adjusted locally in the CNS during development and under physiological and/or pathological conditions by controlling the synthesis of endogenous 5α-reduced neurosteroids.
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Postsynaptic Density 95 controls AMPA Receptor Incorporation during Long-Term Potentiation and Experience-Driven Synaptic Plasticity

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The regulated delivery of AMPA-type glutamate receptors (AMPARs) to synapses is an important mechanism underlying synaptic plasticity. Here, we ask whether the synaptic scaffolding protein PSD-95 (postsynaptic density 95)AQ: B participates in AMPAR incorporation during two forms of synaptic plasticity. In hippocampal slice cultures, the expression of PSD-95– green fluorescent proteinAQ: C (PSD-95–GFP) increases AMPAR currents by selectively delivering glutamate receptor 1AQ: D (GluR1)-containing receptors to synapses, thus mimicking long-term potentiation (LTP). Mutational analysis shows that the N terminal of PSD-95 including the first two PDZ [PSD-95/Discs large (Dlg)/zona occludens-1 (ZO-1)] domains is necessary and sufficient to mediate this effect. Further supporting a role in synaptic plasticity, wild-typeAQ: E PSD-95 occludes LTP and dominant negative forms block LTP. Moreover, we demonstrate that PSD-95 also participates in AMPAR delivery during experience-driven plasticity *in vivo*. In the barrel cortex from experience-deprived animals, the expression of PSD-95–GFP selectively increases AMPAR currents, mimicking experience-driven plasticity. In nondeprived animals, PSD-95–GFP produces no additional potentiation, indicating common mechanisms between PSD-95-mediated potentiation and experience-driven synaptic strengthening. A dominant negative form of PSD-95 blocks experience-driven potentiation of synapses. Pharmacological analysis in slice cultures reveals that PSD-95 acts downstream of other signaling pathways involved in LTP. We conclude that PSD-95 controls activity-dependent AMPAR incorporation at synapses via PDZ interactions not only during LTP *in vitro* but also during experience-driven synaptic strengthening by natural stimuli *in vivo*.
The Journal of Neuroscience, January 28, 2004 • 24(4):916–927

Dopamine Neurons Mediate a Fast Excitatory Signal via Their Glutamatergic Synapses

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Dopamine neurons are thought to convey a fast, incentive salience signal, faster than can be mediated by dopamine. A resolution of this paradox may be that midbrain dopamine neurons exert fast excitatory actions. Using transgenic mice with fluorescent dopamine neurons, in which the axonal projections of the neurons are visible, we made horizontal brain slices encompassing the mesoaccumbens dopamine projection. Focal extracellular stimulation of dopamine neurons in the ventral tegmental area evoked dopamine release and early monosynaptic and late polysynaptic excitatory responses in postsynaptic nucleus accumbens neurons. Local superfusion of the ventral tegmental area with glutamate, which should activate dopamine neurons selectively, produced an increase in excitatory synaptic events. Local superfusion of the ventral tegmental area with the D2 agonist quinpirole, which should increase the threshold for dopamine neuron activation, inhibited the early response. So dopamine neurons make glutamatergic synaptic connections to accumbens neurons. We propose that dopamine neuron glutamatergic transmission may be the initial component of the incentive salience signal.
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DEVELOPMENT/PLASTICITY/REPAIR

Repulsive Guidance Molecule (RGM) Gene Function Is Required for Neural Tube Closure But Not Retinal Topography in the Mouse Visual System

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The establishment of topographic projections in the developing visual system depends on the spatially and temporally controlled expression of axon guidance molecules. In the developing chick tectum, the graded expression of the repulsive guidance molecule (RGM) has been proposed to be involved in controlling the topography of the retinal ganglion cell (RGC) axon termination zones along the anteroposterior axis of the tectum. We now show that there are three mouse proteins homologous to chick RGM displaying similar proteolytic processing but exhibiting differential cell-surface targeting by glycosyl phosphatidylinositolAQ: B anchor addition. Two members of this gene family (*mRGMa* and *mRGMb*) are expressed in complementary patterns in the nervous system, and *mRGMa* is expressed prominently in the superior colliculus at the time of anteroposterior targeting of RGC axons. The third member of the family (*mRGMc*) is expressed almost exclusively in skeletal muscles. Functional studies in the mouse reveal a role for *mRGMa* in controlling cephalic neural tube closure, thus defining an unexpected role for *mRGMa* in early embryonic development. In contrast, *mRGMa* mutant mice did not exhibit defects in anteroposterior targeting of RGC axons to their stereotypic termination zones in the superior colliculus.
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Semaphorin3A Inhibits Nerve Growth Factor-Induced Sprouting of Nociceptive Afferents in Adult Rat Spinal Cord

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Increased expression of NGF after spinal cord injury induces sprouting of primary nociceptive axons. Exogenous application of NGF also results in extensive sprouting of these axons and causes chronic pain in uninjured animals. During development, semaphorin3A is thought to act as a repulsive guidance cue for NGF-responsive nociceptive afferents, restricting their projections to the superficial dorsal horn. We investigated the ability of semaphorin3A to selectively reduce NGF-induced sprouting and neuropathic pain in adult rats. The chemorepulsive effect of virus-mediated semaphorin3A expression was shown to counteract the sprouting induced by NGF in a dose-dependent manner, both *in vitro* and in adult rat spinal cords. Coexpression of semaphorin3A and NGF at moderate to low concentrations within the adult spinal cord reduced sprouting of calcitonin gene-related peptide and substance P-containing axons compared with GFP and NGF coexpression controls. At high expression levels of NGF, there was no difference in sprouting between the semaphorin3A-treated and control groups. The distribution of endogenous primary nociceptive afferents in the spinal cord appeared to be unaffected by semaphorin3A treatment in these experiments. Behavioral assessment shows that semaphorin3A coexpression with NGF led to decreased mechanical allodynia but no significant reductions in thermal hyperalgesia. These findings demonstrate directly that mature sensory afferents maintain their responsiveness to semaphorin3A, suggesting that this molecule might be used therapeutically to control aberrant sensory sprouting involved in pain or autonomic dysfunction.

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BEHAVIORAL/SYSTEMS/COGNITIVE

Prefrontal Cortical–Ventral Striatal Interactions Involved in Affective Modulation of Attentional Performance: Implications for Corticostriatal Circuit Function

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Anatomically segregated systems linking the frontal cortex and the striatum are involved in various aspects of cognitive, affective, and motor processing. In this study, we examined the effects of combined unilateral lesions of the medial prefrontal cortex (mPFC) and the core subregion of the nucleus accumbens (AcbC) in opposite hemispheres (disconnection) on a continuous performance, visual attention test [five-choice serial reaction-time task (5CSRTT)]. The disconnection lesion produced a set of specific changes in performance of the 5CSRTT, resembling changes that followed bilateral AcbC lesions while, in addition, comprising a subset of the behavioral changes after bilateral mPFC lesions previously reported using the same task. Specifically, both mPFC/AcbC disconnection and bilateral AcbC lesions markedly affected aspects of response control related to affective feedback, as indexed by perseverative responding in the 5CSRTT. These effects were comparable, although not identical, to those in animals with either bilateral AcbC or mPFC/AcbC disconnection lesions. The mPFC/AcbC disconnection resulted in a behavioral profile largely distinct from that produced by disconnection of a similar circuit described previously, between the mPFC and the dorsomedial striatum, which were shown to form a functional network underlying aspects of visual attention and attention to action. This distinction provides an insight into the functional specialization of corticostriatal circuits in similar behavioral contexts.

The Journal of Neuroscience, January 28, 2004 • 24(4):773–780

A Circadian Rhythm in the Expression of PERIOD2 Protein Reveals a Novel SCN-Controlled Oscillator in the Oval Nucleus of the Bed Nucleus of the Stria Terminalis

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Circadian rhythms in mammals are regulated not only globally by the master clock in the suprachiasmatic nucleus (SCN), but also locally by widely distributed populations of clock cells in the brain and periphery that control tissue-specific rhythmic outputs. Here we show that the oval nucleus of the bed nucleus of the stria terminalis (BNST-OV) exhibits a robust circadian rhythm in expression of the Period2 (PER2) clock protein.AQ: A PER2 expression is rhythmic in the BNST-OV in rats housed under a light/dark cycle or in constant darkness, in blind rats, and in mice, and is in perfect synchrony with the PER2 rhythm of the SCN. Constant light or bilateral SCN lesions abolish the rhythm of PER2 in the BNST-OV. Large abrupt shifts in the light schedule transiently uncouple the BNST-OV rhythm from that of the SCN. Re-entrainment of the PER2 rhythm is faster in the SCN than in the BNST-OV, and it is faster after a delay than an advance shift. Bilateral adrenalectomy blunts the PER2 rhythm in the BNST-OV. Thus, the BNST-OV contains circadian clock cells that normally oscillate in synchrony with the SCN, but these cells appear to require both input from the SCN and circulating glucocorticoids to maintain their circadian oscillation. Taken together with what is known about the functional organization of the connections of the BNST-OV with systems of the brain involved in stress and motivational processes, these findings place BNST-OV oscillators in a position to influence specific physiological and behavioral rhythms downstream from the SCN clock.

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Phox2a Gene, A6 Neurons, and Noradrenaline Are Essential for Development of Normal Respiratory Rhythm in Mice

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Although respiration is vital to the survival of all mammals from the moment of birth, little is known about the genetic factors controlling the prenatal maturation of this physiological process. Here we investigated the role of the *Phox2a*AQ: E gene that encodes for a homeodomain protein involved in the generation of noradrenergic A6 neurons in the maturation of the respiratory network. First, comparisons of the respiratory activity of fetuses delivered surgically from heterozygous *Phox2a* pregnant mice on gestational day 18 showed that the mutants had impaired *in vivo* ventilation, *in vitro* respiratory-like activity, and *in vitro* respiratory responses to central hypoxia and noradrenaline. Second, pharmacological studies on wild-type neonates showed that endogenous noradrenaline released from pontine A6 neurons potentiates rhythmic respiratory activity via α1 medullary adrenoceptors. Third, transynapticAQ: F tracing experiments in which rabies virus was injected into the diaphragm confirmed that A6 neurons were connected to the neonatal respiratory network. Fourth, blocking the α1 adrenoceptors in wild-type dams during late gestation with daily injections of the α1 adrenoceptor antagonist prazosin induced *in vivo* and *in vitro* neonatal respiratory deficits similar to those observed in *Phox2a* mutants. These results suggest that noradrenaline, A6 neurons, and the *Phox2a* gene, which is crucial for the generation of A6 neurons, are essential for development of normal respiratory rhythm in neonatal mice. Metabolic noradrenaline disorders occurring during gestation therefore may induce neonatal respiratory deficits, in agreement with the catecholamine anomalies reported in victims of sudden infant death syndrome.

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Polymorphisms in the Taste Receptor Gene (*Tas1r3*) Region Are Associated with Saccharin Preference in 30 Mouse Strains

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The results of recent studies suggest that the mouse *Sac* (saccharin preference) locus is identical to the *Tas1r3* (taste receptor) gene. The goal of this study was to identify *Tas1r3* sequence variants associated with saccharin preference in a large number of inbred mouse strains. Initially, we sequenced ~6.7 kb of the *Tas1r3* gene and its flanking regions from six inbred mouse strains with high and low saccharin preference, including the strains in which the *Sac* alleles were described originally (C57BL/6J, Sac^b; DBA/2J, Sac^d). Of the 89 sequence variants detected among these six strains, eight polymorphic sites were significantly associated with preferences for 1.6 mM saccharin. Next, each of these eight variant sites were genotyped in 24 additional mouse strains. Analysis of the genotype–phenotype associations in all 30 strains showed the strongest association with saccharin preference at three sites: nucleotide (nt) −791 (3 bp insertion/deletion), nt +135 (Ser45Ser), and nt +179 (Ile60Thr). We measured *Tas1r3* gene expression, transcript size, and T1R3 immunoreactivity in the taste tissue of two inbred mouse strains with different *Tas1r3* haplotypes and saccharin preferences. The results of these experiments suggest that the polymorphisms associated with saccharin preference do not act by blocking gene expression, changing alternative splicing, or interfering with protein translation in taste tissue. The amino acid substitution (Ile60Thr) may influence the ability of the protein to form dimers or bind sweeteners. Here, we present data for future studies directed to experimentally confirm the function of these polymorphisms and highlight some of the difficulties of identifying specific DNA sequence variants that underlie quantitative trait loci.

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Estrogen-Induced μ-Opioid Receptor Internalization in the Medial Preoptic Nucleus Is Mediated via Neuropeptide Y-Y_1 Receptor Activation in the Arcuate Nucleus of Female Rats

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The endogenous peptides β-endorphin (β-END) and neuropeptide Y (NPY) have been implicated in regulating sexual receptivity. Both β-END and NPY systems are activated by estrogen and inhibit female sexual receptivity. The initial estrogen-induced sexual nonreceptivity is correlated with the activation and internalization of μ-opioid receptors (MORs), in the medial preoptic nucleus (MPN). Progesterone reverses the estrogen-induced activation/internalization of MOR and induces the sexual receptive behavior lordosis. To determine whether NPY and endogenous opioids interact, we tested the hypothesis that estrogen-induced MOR activation is mediated through NPY-Y_1 receptor (Y_1R) activation. Retrograde tract tracing demonstrated Y_1R on β-END neurons that projected to the MPN. Sex steroid modulation of MOR in the MPN acts through NPY and the Y_1R. Estradiol administration or intracerebroventricular injection of NPY activated/internalized Y_1R in the arcuate nucleus and MOR in the MPN of ovariectomized (OVX) rats. Moreover, the selective Y_1R agonist [Leu31, Pro34]-Neuropeptide Y (LPNY) internalized MOR in the MPN of OVX rats. The Y_1R antagonist (Cys^{31}, Nva^{34})-Neuropeptide Y $(27–36)_2$ prevented estrogen-induced Y_1R and MOR activation/internalization. NPY reversed the progesterone blockade of estradiol-induced Y_1R and MOR internalization in the arcuate nucleus and MPN, respectively. Behaviorally, LPNY inhibited estrogen plus progesterone-induced lordosis,

and the MOR-selective antagonist D-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr amide reversed LPNY-induced inhibition of lordosis. These results suggest that a sequential sex steroid activation of NPY and MOR circuits regulates sexual receptivity.

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Shrinkage of the Entorhinal Cortex over Five Years Predicts Memory Performance in Healthy Adults

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Lesions in the hippocampus (HC),AQ: A the entorhinal cortex (EC), and the prefrontal cortex (PFC) are associated with impairment of episodic memory; reduced HC volume is linked to memory declines in dementia; and decline in EC volume predicts progression from mild cognitive impairment to dementia. However, in healthy adults, the relationship between memory and regional volumes is unclear, and no data are available on the relationship of longitudinal regional shrinkage to memory performance in a cognitively intact population. The objective of this study was to examine whether shrinkage of the EC, HC, and PFC over a 5 year period can predict declarative memory performance in healthy adults. The volumes of three brain regions were measured on magnetic resonance images that were acquired twice, 5 years apart. Multiple measures of episodic memory were administered at follow-up. Results indicated that the volume of HC and PFC (but not EC) correlated with age at baseline and follow-up. However, after age differences in memory were taken into account, none of the regional volumes was associated with memory performance at follow-up. In contrast, greater annual rate of shrinkage in EC (but not HC or PFC) predicted poorer memory performance. Thus, in a healthy and educated population, even mild age-related shrinkage of the EC may be a sensitive predictor of memory decline.

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Perceptual Correlates of Nociceptive Long-Term Potentiation and Long-Term Depression in Humans

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Long-term potentiation (LTP) and long-term depression (LTD) of synaptic strength are ubiquitous mechanisms of synaptic plasticity, but their functional relevance in humans remains obscure. Here we report that a long-term increase in perceived pain to electrical test stimuli was induced by high-frequency electrical stimulation (HFS) (5×1 sec at 100 Hz) of peptidergic cutaneous afferents (27% above baseline, undiminished for >3 hr). In contrast, a long-term decrease in perceived pain (27% below baseline, undiminished for 1 hr) was induced by low-frequency stimulation (LFS) (17 min at 1 Hz). Pain testing with punctate mechanical probes (200 μm diameter) in skin adjacent to the HFS–AQ: ALFS conditioning skin site revealed a marked twofold to threefold increase in pain sensitivity (secondary hyperalgesia, undiminished for >3 hr) after HFS but also a moderate secondary hyperalgesia (30% above baseline) after strong LFS. Additionally, HFS but not LFS caused pain to light tactile stimuli in adjacent skin (allodynia). In summary, HFS and LFS stimulus protocols that induce LTP or LTD in spinal nociceptive pathways in animal experiments led to similar LTP- and LTD-like changes in human pain perception (long-term hyperalgesia or hypoalgesia) mediated by the conditioned pathway. Additionally, secondary hyperalgesia and allodynia in adjacent skin induced by the HFS protocol and, to a minor extent, also by the LFS protocol, suggested that these perceptual changes encompassed an LTP-like heterosynaptic facilitation of adjacent nociceptive pathways by a hitherto unknown mechanism.

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Morphological Effects of Estrogen on Cholinergic Neurons *In Vitro* Involves Activation of Extracellular Signal-Regulated Kinases

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In the present study, we examined the ability of estrogen to enhance cholinergic neurite arborization *in vitro* and identified the signal transduction cascade associated with this effect. Basal forebrain primordia collected from rat pups on postnatal day 1 were cultured for 2 weeks and then treated with 5 nM 17β-estradiol for 24 hr. Cholinergic neurons were identified immunocytochemically with an antibody against the vesicular acetylcholine transporter and digitally photographed. Morphological analysis indicated that female cultures respond to estrogen treatment with an increase in total neurite length per neuron (4.5-fold over untreated controls) and in total branch segment number per neuron (2.3-fold over controls). In contrast, there was no change in total neurite length per neuron in male cultures, and we also observed a decrease in total branch segment number per neuron (0.5-fold below controls). Detailed histograms indicated that estrogen increases primary and secondary branch length and number and also increases terminal neuritic branches to the seventh order in female cultures. In a second set of experiments, we investigated the signal transduction cascade involved in this response, and found that an upstream extracellular signal-regulated kinase (ERK) inhibitor blocked the ability of estrogen to enhance outgrowth in female cultures. Our study provides strong evidence in support of the fact that the ERK pathway is required for estrogen-induced structural plasticity in the cholinergic system of female rats. Understanding the intracellular processes that underlie the response of cholinergic neurons to estrogen provides a necessary step in elucidating how cholinergic neurons can be particularly susceptible to degeneration in postmenopausal women.

The Journal of Neuroscience, January 28, 2004 • 24(4):982–990

Endogenous Activation of mGlu5 Metabotropic Glutamate Receptors Contributes to the Development of Nigro-Striatal Damage Induced by 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine in Mice

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AQ: BWe combined the use of knock-out mice and subtype-selective antagonists [2-methyl-6-(phenylethynyl)pyridine (MPEP) and (E)-2-methyl-6-(2-phenylethenyl)-pyridine (SIB1893)]AQ: C to examine whether endogenous activation of mGlu5 metabotropic glutamate receptors contributes to the pathophysiology of nigro-striatal damage in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of parkinsonism. High doses of MPTP (four injections of AQ: D20 mg/kg, i.p., every 2 hr) induced a high mortality rate and a nearly total degeneration of the nigro-striatal pathway in wild-type mice. mGlu5 knock-outAQ: E mice were less sensitive to MPTP toxicity, as shown by a higher survival and a milder nigro-striatal damage. Protection against MPTP (80 mg/kg) toxicity was also observed after MPEP injections (four injections of 5 mg/kg, i.p., 30 min before each MPTP injection). MPEP treatment did not further increase neuroprotection against 80 mg/kg of MPTP in mGlu5 knock-out mice, indicating that the drug acted by inhibiting mGlu5 receptors. In wild-type mice, MPEP was also neuroprotective when challenged against lower doses of MPTP (either 30 mg/kg, single injection, or four of 10 mg/kg injections). The action of MPEP was mimicked by SIB1893 but not by the mGlu1 receptor antagonist 7-hydroxyiminocyclopropan[b]chromen-1a-carboxylic acid ethyl ester. MPEP did not change the kinetics of 1-methyl-4-phenylpyridinium ion formation in the striatum of mice injected with MPTP. We conclude that mGlu5 receptors act as amplifiers of MPTP toxicity and that mGlu5 receptor antagonists may limit the extent of nigro-striatal damage in experimental models of parkinsonism.

The Journal of Neuroscience, January 28, 2004 • 24(4):828–835

Enhanced *In Vitro* Midbrain Dopamine Neuron Differentiation, Dopaminergic Function, Neurite Outgrowth, and 1-Methyl-4-Phenylpyridium Resistance in Mouse Embryonic Stem Cells Overexpressing Bcl-XL

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Embryonic stem (ES) cells provide a potentially unlimited source of specialized cells for regenerative medicine. The ease of inducing stable genetic modifications in ES cells allows for *in vitro* manipulations to enhance differentiation into specific cell types and to optimize *in vivo* function of differentiated progeny in animal models of disease. We have generated mouse ES cells that constitutively express Bcl-XL, an antiapoptotic protein of Bcl-2 family. *In vitro* differentiation of Bcl-XL overexpressing ES (Bcl-ES) cells resulted in higher expression of genes related to midbrain dopamine (DA) neuron development and increased the number of ES-derived neurons expressing midbrain DA markers compared with differentiation of wild-type ES cells. Moreover, DA neurons derived from Bcl-ES cells were less susceptible to 1-methyl-4-phenylpyridium, a neurotoxin for DA neurons. On transplantation into parkinsonian rats, the Bcl-ES-derived DA neurons exhibited more extensive fiber outgrowth and led to a more pronounced reversal of behavioral symptoms than wild-type ES-derived DA neurons. These data suggest a role for Bcl-XL during *in vitro* midbrain DA neuron differentiation and provide an improved system for cell transplantation in a preclinical animal model of Parkinson's disease.

The Journal of Neuroscience, January 28, 2004 • 24(4):843–852

Translamellar Disinhibition in the Rat Hippocampal Dentate Gyrus after Seizure-Induced Degeneration of Vulnerable Hilar Neurons

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Longitudinally restricted axonal projections of hippocampal granule cells suggest that transverse segments of the granule cell layer may operate independently (the "lamellar" hypothesis). Longitudinal projections of excitatory hilar mossy cells could be viewed as antithetical to lamellar function, but only if longitudinal impulse flow effectively excites distant granule cells. We, therefore, determined the effect of focal granule cell discharges on granule cells located >2 mm along the longitudinal axis. During perforant pathway stimulation in urethane-anesthetized rats, passive diffusion of the $GABA_A$ receptor antagonist bicuculline methiodide from the tip of a glass recording electrode evoked granule cell discharges and c-Fos expression in granule cells, mossy cells, and inhibitory interneurons, within a ~400 μm radius. This focally evoked activity powerfully suppressed distant granule cell-evoked responses recorded simultaneously ~2.5–4.5 mm longitudinally. Three days after kainic acid-induced

status epilepticus or prolonged perforant pathway stimulation, translamellar inhibition was intact in rats with <40% hilar neuron loss but was consistently abolished after extensive (>85%) hilar cell loss. Retrograde transport of Fluoro-Gold (FG) from the rostral dentate gyrus revealed that few inhibitory interneurons were among the many retrogradely labeled hilar neurons 2.5–4.5 mm longitudinally. Although many somatostatin-positive hilar interneurons effectively transported FG from the distant septum, few of these neurons transported detectable FG from much closer hippocampal injection sites. Inhibitory basket and chandelier cells also exhibited minimal longitudinal FG transport. These findings suggest that translamellar disinhibition may result from the loss of vulnerable, longitudinally projecting mossy cells and may represent a network-level mechanism underlying postinjury hippocampal dysfunction and epileptic network hyperexcitability.
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Presynaptic Localization of Neprilysin Contributes to Efficient Clearance of Amyloid-β Peptide in Mouse Brain

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A local increase in amyloid-β peptide (Aβ) is closely associated with synaptic dysfunction in the brain in Alzheimer's disease. Here, we report on the catabolic mechanism of Aβ at the presynaptic sites. Neprilysin, an Aβ-degrading enzyme, expressed by recombinant adeno-associated viral vector-mediated gene transfer, was axonally transported to presynaptic sites through afferent projections of neuronal circuits. This gene transfer abolished the increase in Aβ levels in the hippocampal formations of neprilysin-deficient mice and also reduced the increase in young mutant amyloid precursor protein transgenic mice. In the latter case, Aβ levels in the hippocampal formation contralateral to the vector-injected side were also significantly reduced as a result of transport of neprilysin from the ipsilateral side, and in both sides soluble Aβ was degraded more efficiently than insoluble Aβ. Furthermore, amyloid deposition in aged mutant amyloid precursor protein transgenic mice was remarkably decelerated. Thus, presynaptic neprilysin has been demonstrated to degrade Aβ efficiently and to retard development of amyloid pathology.
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Spinal Cord Injury Techniques & Technologies

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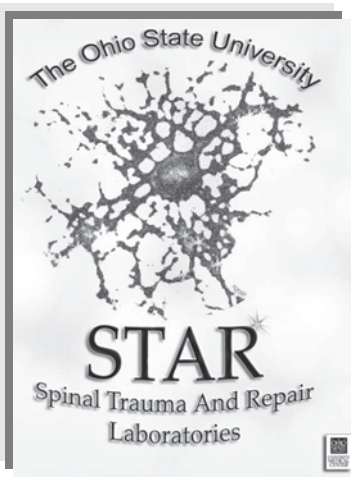

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