

This Week in The Journal

● Cellular/Molecular

GluR1 Delivery in Mental Retardation

Hailan Hu, Yi Qin, Genrieta Bochorishvili, Yinghua Zhu, Linda van Aelst, and J. Julius Zhu

(see pages 7847–7862)

Fragile X mental retardation protein (FMRP) regulates many proteins, including Ras small GTPases, which are required for targeting of AMPA receptors to synapses during synaptic enhancement. Loss of FMRP causes mental retardation in humans, but experiments in which its gene, *FMR1*, was knocked out in mice have produced variable effects. Now Hu et al. have expressed mutant AMPA receptor subunits [glutamate receptors (GluRs)] in hippocampal and cortical neurons to track synaptic delivery in wild-type and *FMR1*-null mice. LTP was reduced by 50% in hippocampal slices from *FMR1* knock-outs, likely due to defective trafficking of GluR1. The neuromodulator histamine normally increases synaptic delivery of GluRs, in part via activation of a kinase pathway involving Ras, phosphoinositide 3-kinase, and protein kinase B, which phosphorylates GluR1. This signaling pathway is disrupted in *FMR1* knock-outs, but overexpression of Ras or reducing the activation threshold of its downstream kinases restored LTP and synaptic delivery of GluR1.

▲ Development/Plasticity/Repair

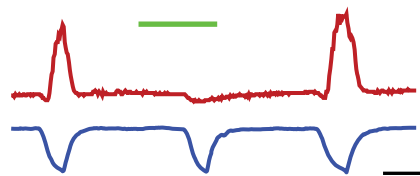
TRPA1-Mediated Cold Sensitivity

Otto Fajardo, Victor Meseguer, Carlos Belmonte, and Félix Viana

(see pages 7863–7875)

Although TRPA1 channels are activated by cold in cell lines, whether they have a physiologically relevant role in neurons has been questioned. Now the controversy appears to have been settled by Fajardo et al. Approximately half of visceral afferent neurons cultured from rat nodose ganglion responded to cold, as indicated by calcium imaging. Responses in the

presence of specific agonists and antagonists of TRPA1 and TRPM8 (the main cold receptor in somatosensory neurons of the dorsal root ganglion) indicated that TRPA1 is responsible for most of the cold sensitivity in the nodose system. Interestingly, a subset of cold-sensitive nodose neurons were TRPA1 independent. Although mice had a smaller percentage of cold-sensitive nodose neurons than rats, TRPA1 knock-out eliminated most of the sensitivity, indicating that TRPA1 is also the main cold-responsive receptor in mouse visceral afferents. Some TRPA1-dependent cold-sensitive nodose neurons innervate the larynx, likely mediating reflexive responses to cold temperatures, such as coughing.



Response of a rat nodose neuron (red trace) to decreasing temperature (blue trace) is inhibited by application of a TRPA1 antagonist (green bar). Horizontal scale, 3 min; vertical scale, 200 nM Δ [Ca] or $\sim 25^\circ\text{C}$. See the article by Fajardo et al. for details.

■ Behavioral/Systems/Cognitive

Dissociating Pain From Its Negative Affect

Satoshi Deyama, Takahiro Katayama, Atsushi Ohno, Takayuki Nakagawa, Shuji Kaneko, Taku Yamaguchi, Mitsuhiro Yoshioka, and Masabumi Minami

(see pages 7728–7736)

The negative affective states produced by pain can be reduced without eliminating the pain itself, according to Deyama et al. When formalin is injected into a rat's paw, the rat displays signs of pain (licking and biting the paw) and of aversion (the rat is conditioned to avoid the place where it experienced the pain). Deyama et al. found that formalin injection increased noradrenaline levels in the bed nucleus of

the stria terminalis (BNST), a brain region implicated in negative affective states such as anxiety and fear. Injecting β_2 -adrenoreceptor blockers into the BNST decreased the conditioned place aversion, but had no effect on paw licking and biting. Similarly, inhibiting protein kinase A (which normally increases after β -adrenoreceptor activation) reduced conditioned place aversion without altering pain behaviors. Conversely, injecting β -adrenoreceptor agonist into the BNST induced conditioned place aversion, indicating a prominent role for this pathway in producing the negative affective component of pain.

◆ Neurobiology of Disease

Dopamine and Noradrenaline Interactions in Learning

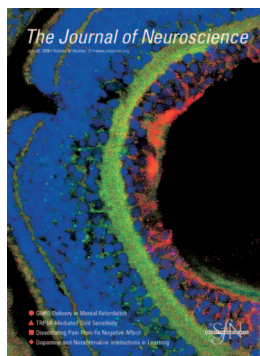
Liselijn A. B. Wisman, Gurdal Sahin, Matthew Maingay, Giampiero Leanza, and Deniz Kirik

(see pages 7797–7807)

This week, Wisman et al. describe interactions between dopaminergic and cholinergic pathways in modulating spatial memory. The authors used selective neurotoxins to lesion mesocorticolimbic dopaminergic neurons in the ventral tegmental area (VTA), septohippocampal cholinergic neurons in the medial septum, and/or basolateral cholinergic neurons in the nucleus basalis magnocellularis. They then tested various forms of spatial learning using Morris water maze tasks. Reference memory—in which rats learned over several days to use extramaze navigational cues to find a stationary platform—was disrupted by dopaminergic lesions, but unaffected by cholinergic lesions. In contrast, performance on a working memory task—in which rats had to relearn the position of a platform that was moved daily—was not disrupted by dopamine or cholinergic lesions alone, but was disrupted when dopaminergic lesions were paired with septohippocampal cholinergic lesions. The results suggest that loss of dopaminergic neurons in the VTA contribute to cognitive decline in Parkinson's disease patients.

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Cover legend: Expression of marker proteins in the retina of zebrafish embryos (72 h postfertilization). Frozen eye sections were stained with PKC β 1 (green), Zn-5 (red), and DAPI (blue). For more information, see the article by Nakaya et al. in this issue (pages 7900–7910).

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Articles

CELLULAR/MOLECULAR

Analog Modulation of Mossy Fiber Transmission Is Uncoupled from Changes in Presynaptic Ca^{2+}

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Subthreshold somatic depolarization has been shown recently to modulate presynaptic neurotransmitter release in cortical neurons. To understand the mechanisms underlying this mode of signaling in the axons of dentate granule cells (hippocampal mossy fibers), we have combined two-photon Ca^{2+} imaging with dual-patch recordings from somata and giant boutons forming synapses on CA3 pyramidal cells. In intact axons, subthreshold depolarization propagates both orthodromically and antidromically, with an estimated length constant of 200–600 μm depending on the signal waveform. Surprisingly, presynaptic depolarization sufficient to enhance glutamate release at mossy fiber–CA3 pyramidal cell synapses has no detectable effect on either basal Ca^{2+} -dependent fluorescence or action-potential-evoked fluorescence transients in giant boutons. We further estimate that neurotransmitter release varies with presynaptic Ca^{2+} entry with a 2.5-power relationship and that depolarization-induced synaptic facilitation remains intact in the presence of high-affinity presynaptic Ca^{2+} buffers or after blockade of local Ca^{2+} stores. We conclude that depolarization-dependent modulation of transmission at these boutons does not rely on changes in presynaptic Ca^{2+} .

The Journal of Neuroscience, July 30, 2008 • 28(31):7765–7773

The Interface between Extracellular and Transmembrane Domains of Homomeric Cys-Loop Receptors Governs Open-Channel Lifetime and Rate of Desensitization

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The lifetimes of activated postsynaptic receptor channels contribute to the efficiency of synaptic transmission. Here we show that structural differences within the interface dividing extracellular and transmembrane domains of homomeric $\alpha 7$ and 5-HT_{3A} receptors account for the large differences in open-channel lifetime and time of desensitization onset between these contrasting members of the Cys-loop receptor superfamily. For $\alpha 7$ receptors, agonist-evoked single-channel currents appear mainly as isolated brief openings ($\tau_o = 0.35$ ms), whereas macroscopic currents after a step pulse of agonist desensitize rapidly ($\tau_d = 0.4$ ms). In contrast for 5-HT_{3A} receptors, agonist-evoked single-channel currents appear as clusters of many long openings in quick succession ($\tau_{\text{cluster}} = 1.2$ s), whereas macroscopic currents desensitize slowly ($\tau_d = 1.1$ s). A chimeric $\alpha 7$ -5HT_{3A} receptor exhibits functional properties intermediate between those of the parent receptors, but the functional signatures of each parent are reconstituted after substituting the major loops within the interface of the extracellular and transmembrane domains from the corresponding parent receptor. Furthermore, these structural loops contribute to open-channel lifetime and time of desensitization onset in a nonadditive manner. The results suggest that desensitization is the major determinant of the lifetimes of activated $\alpha 7$ and 5-HT_{3A} receptors and that functional differences between the two receptors arise primarily through structural differences at the interface between extracellular and transmembrane domains.

The Journal of Neuroscience, July 30, 2008 • 28(31):7808–7819

PKM ζ Maintains Late Long-Term Potentiation by *N*-Ethylmaleimide-Sensitive Factor/GluR2-Dependent Trafficking of Postsynaptic AMPA Receptors

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Although the maintenance mechanism of late long-term potentiation (LTP) is critical for the storage of long-term memory, the expression mechanism of synaptic enhancement during late-LTP is unknown. The autonomously active protein kinase C isoform, protein kinase M ζ (PKM ζ), is a core molecule maintaining late-LTP. Here we show that PKM ζ maintains late-LTP through persistent *N*-ethylmaleimide-sensitive factor (NSF)/glutamate receptor subunit 2 (GluR2)-dependent trafficking of AMPA receptors (AMPA receptors) to the synapse. Intracellular perfusion of PKM ζ into CA1 pyramidal cells causes potentiation of postsynaptic AMPAR responses; this synaptic enhancement is mediated through NSF/GluR2 interactions but not vesicle-associated membrane protein-dependent exocytosis. PKM ζ may act through NSF to release GluR2-containing receptors from a reserve pool held at extrasynaptic sites by protein interacting with C-kinase 1 (PICK1), because disrupting GluR2/PICK1 interactions mimic and occlude PKM ζ -mediated AMPAR potentiation. During LTP maintenance, PKM ζ directs AMPAR trafficking, as measured by NSF/GluR2-dependent increases of GluR2/3-containing receptors in synaptosomal fractions from tetanized slices. Blocking this trafficking mechanism reverses established late-LTP and persistent potenti-

ation at synapses that have undergone synaptic tagging and capture. Thus, PKM ζ maintains late-LTP by persistently modifying NSF/GluR2-dependent AMPAR trafficking to favor receptor insertion into postsynaptic sites.

The Journal of Neuroscience, July 30, 2008 • 28(31):7820–7827

CELLULAR MOLECULAR

Ras Signaling Mechanisms Underlying Impaired GluR1-Dependent Plasticity Associated with Fragile X Syndrome

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Fragile X syndrome, caused by the loss of *FMR1* gene function and loss of fragile X mental retardation protein (FMRP), is the most commonly inherited form of mental retardation. The syndrome is characterized by associative learning deficits, reduced risk of cancer, dendritic spine dysmorphogenesis, and facial dysmorphism. However, the molecular mechanism that links loss of function of *FMR1* to the learning disability remains unclear. Here, we report an examination of small GTPase Ras signaling and synaptic AMPA receptor (AMPA-R) trafficking in cultured slices and intact brains of wild-type and *FMR1* knock-out mice. In *FMR1* knock-out mice, synaptic delivery of GluR1-, but not GluR2L- and GluR4-containing AMPA-Rs is impaired, resulting in a selective loss of GluR1-dependent long-term synaptic potentiation (LTP). Although Ras activity is upregulated, its downstream MEK (extracellular signal-regulated kinase kinase)–ERK (extracellular signal-regulated kinase) signaling appears normal, and phosphoinositide 3-kinase (PI3K)–protein kinase B (PKB; or Akt) signaling is compromised in *FMR1* knock-out mice. Enhancing Ras–PI3K–PKB signaling restores synaptic delivery of GluR1-containing AMPA-Rs and normal LTP in *FMR1* knock-out mice. These results suggest aberrant Ras signaling as a novel mechanism for fragile X syndrome and indicate manipulating Ras–PI3K–PKB signaling to be a potentially effective approach for treating patients with fragile X syndrome.

The Journal of Neuroscience, July 30, 2008 • 28(31):7847–7862

Articles

CELLULAR/MOLECULAR

Zebrafish Olfactomedin 1 Regulates Retinal Axon Elongation *In Vivo* and Is a Modulator of Wnt Signaling Pathway

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Olfactomedin 1 (Olfm1) is a secreted glycoprotein belonging to a family of olfactomedin domain-containing proteins. It is involved in the regulation of neural crest production in chicken and promotes neuronal differentiation in *Xenopus*. Here, we investigate the functions of Olfm1 in zebrafish eye development. Overexpression of full-length Olfm1, and especially its BMY form lacking the olfactomedin domain, increased the thickness of the optic nerve and produced a more extended projection field in the optic tectum compared with control embryos. In contrast, injection of *olfm1*–morpholino oligonucleotide (Olfm1–MO) reduced the eye size, inhibited optic nerve extension, and increased the number of apoptotic cells in the retinal ganglion cell and inner nuclear layers. Overexpression of full-length Olfm1 increased the lateral separation of the expression domains of eye-field markers, *rx3* and *six3*. The Olfm1–MO had the opposite effect. These data suggest that zebrafish Olfm1 may play roles in the early eye determination, differentiation, optic nerve extension, and branching of the retinal ganglion cell axon terminals, with the N-terminal region of Olfm1 being critical for these effects. Injection of RNA encoding WIF-1, a secreted inhibitor of Wnt signaling, caused changes in the expression pattern of *rx3* similar to those observed after Olfm1–MO injection. Simultaneous overexpression of WIF-1 and Olfm1 abolished the WIF-1 effect. Physical interaction of WIF-1 and Olfm1 was demonstrated by coimmunoprecipitation experiments. We concluded that Olfm1 serves as a modulator of Wnt signaling.

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Diverse Mechanisms Underlie Glycinergic Feedback Transmission onto Rod Biopolar Cells in Rat Retina

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Synaptic inhibition shapes visual signaling in the inner retina, but the physiology of most amacrine cells, the interneurons that mediate this inhibition, is poorly understood. Discerning the function of most individual amacrine cell types is a daunting task, because few molecular or morphological markers specifically distinguish between

approximately two dozen different amacrine cell types. Here, we examine a functional subset of amacrine cells by pharmacologically isolating glycinergic inhibition and evoking feedback IPSCs in a single cell type, the rod bipolar cell (RBC), with brief glutamate applications in the inner plexiform layer. We find that glycinergic amacrine cells innervating RBCs receive excitatory inputs from ON and OFF bipolar cells primarily via NMDA receptors (NMDARs) and Ca^{2+} -impermeable AMPA-type glutamate receptors. Glycine release from amacrine cells is triggered by Ca^{2+} influx through both voltage-gated Ca^{2+} (Ca_v) channels and NMDARs. These intracellular Ca^{2+} signals are amplified by Ca^{2+} -induced Ca^{2+} release via both ryanodine and IP_3 receptors, which are activated independently by Ca^{2+} influx through Ca_v channels and NMDARs, respectively. Glycinergic feedback signaling depends strongly, although not completely, on voltage-gated Na^+ channels, and the spatial extent of feedback inhibition is expanded by gap junction connections between glycinergic amacrine cells. These results indicate that a diversity of mechanisms underlie glycinergic feedback inhibition onto RBCs, yet they highlight several physiological themes that appear to distinguish amacrine cell function.

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DEVELOPMENT/PLASTICITY/REPAIR

A Core Paired-Type and POU Homeodomain-Containing Transcription Factor Program Drives Retinal Bipolar Cell Gene Expression

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The diversity of cell types found within the vertebrate CNS arises in part from action of complex transcriptional programs. In the retina, the programs driving diversification of various cell types have not been completely elucidated. To investigate gene regulatory networks that underlie formation and function of one retinal circuit component, the bipolar cell, transcriptional regulation of three bipolar cell-enriched genes was analyzed. Using *in vivo* retinal DNA transfection and reporter gene constructs, a 200 bp *Grm6* enhancer sequence, a 445 bp *Cabp5* promoter sequence, and a 164 bp *Chx10* enhancer sequence, were defined, each driving reporter expression specifically in distinct but overlapping bipolar cell subtypes. Bioinformatic analysis of sequences revealed the presence of potential paired-type and POU homeodomain-containing transcription factor binding sites, which were shown to be critical for reporter expression through deletion studies. The paired-type homeodomain transcription factors (TFs) *Crx* and *Otx2* and the POU homeodomain factor *Brn2* are expressed in bipolar cells and interacted with the predicted binding sequences as assessed by electrophoretic mobility shift assay. *Grm6*, *Cabp5*, and *Chx10* reporter activity was reduced in *Otx2* loss-of-function retinas. Endogenous gene expression of bipolar cell molecular markers was also dependent on paired-type homeodomain-containing TFs, as assessed by RNA *in situ* hybridization and reverse transcription-PCR in mutant retinas. *Cabp5* and *Chx10* reporter expression was reduced in dominant-negative *Brn2*-transfected retinas. The paired-type and POU homeodomain-containing TFs *Otx2* and *Brn2* together appear to play a common role in regulating gene expression in retinal bipolar cells.

The Journal of Neuroscience, July 30, 2008 • 28(31):7748–7764

Facilitation of Stepping with Epidural Stimulation in Spinal Rats: Role of Sensory Input

Igor Lavrov,¹ Grégoire Courtine,⁴ Christine J. Dy,¹ Rubia van den Brand,⁴ Andy J. Fong,⁵ Yuri Gerasimenko,^{1,6} Hui Zhong,¹ Roland R. Roy,^{1,3} and V. Reggie Edgerton^{1,2,3}

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We investigated the role of afferent information during recovery of coordinated rhythmic activity of the hindlimbs in rats with a complete spinal cord section (approximately T8) and unilateral deafferentation (T12–S2) to answer the following questions: (1) Can bilateral stepping be generated with only afferent projections intact on one side? (2) Can the sensory input from the non-deafferented side compensate for the loss of the afferent input from the deafferented side through the crossed connections within the lumbosacral spinal cord? (3) Which afferent projections to the spinal cord from the non-deafferented side predominantly mediate the effect of epidural stimulation to facilitate stepping? Recovery of stepping ability was tested under the facilitating influence of epidural stimulation at the S1 spinal segment, or epidural stimulation plus quipazine, a 5-HT agonist. All chronic spinal rats were able to generate stepping-like patterns on a moving treadmill on the non-deafferented, but not deafferented, side from 3 to 7 weeks after surgery when facilitated by epidural stimulation. Adaptation to the loss of unilateral afferent input was evident at 7 weeks after surgery, when some movements occurred on the deafferented side. Spinal-cord-evoked potentials were observed on both sides, although middle (monosynaptic) and late (long latency) responses were more prominent on the non-deafferented side. The afferent information arising from the non-deafferented side, however, eventually could mediate limited restoration of hindlimb movements on the deafferented side. These data suggest that facilitation of stepping with epidural stimulation is mediated primarily through ipsilateral afferents that project to the locomotor networks.

The Journal of Neuroscience, July 30, 2008 • 28(31):7774–7780

TRPA1 Channels Mediate Cold Temperature Sensing in Mammalian Vagal Sensory Neurons: Pharmacological and Genetic Evidence

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Cold thermoreceptors have been described in different territories of the vagus nerve. Application of cold temperature to these visceral afferents can evoke major protective reflexes and thermoregulatory responses. However, virtually nothing is known about the transduction mechanisms underlying cold sensitivity in vagal afferents. Here, we

investigated the effects of cold stimulation on intracellular calcium responses and excitability of cultured vagal sensory neurons in the rat nodose ganglion. A large fraction of vagal neurons were activated by cold, with a mean threshold of $\sim 24^{\circ}\text{C}$. Cooling was accompanied by development of a small inward current and the firing of action potentials. Most cold-sensitive neurons were also activated by heat and capsaicin, suggesting a nociceptive function. The pharmacological response to TRPM8 and TRPA1 agonists and antagonists suggested that, unlike results observed in somatic tissues, TRPA1 is the major mediator of cold-evoked responses in vagal visceral neurons. Thus, most cold-evoked responses were potentiated by cinnamaldehyde, menthol, icilin, and BCTC [4-(3-chloro-pyridin-2-yl)-piperazine-1-carboxylic acid (4-tert-butyl-phenyl)-amide], agonists of TRPA1, and were inhibited by ruthenium red, camphor, and HC03001 [2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-N-(4-isopropylphenyl)acetamide]. Results in mouse nodose neurons revealed a similar pharmacological profile of cold-evoked responses. Furthermore, experiments in TRPA1 knock-out mice showed a large reduction in the percentage of cold-sensitive neurons compared with wild-type animals. Together, these results support an important role of TRPA1 channels in visceral thermosensation and indicate major differences in the transduction of temperature signals between somatic and visceral sensory neurons. *The Journal of Neuroscience*, July 30, 2008 • 28(31):7863–7875

BEHAVIORAL/SYSTEMS/COGNITIVE

Activation of the β -Adrenoceptor–Protein Kinase A Signaling Pathway within the Ventral Bed Nucleus of the Stria Terminalis Mediates the Negative Affective Component of Pain in Rats

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Pain is an unpleasant sensory and emotional experience. The neural systems underlying the sensory component of pain have been studied extensively, but we are only beginning to understand those underlying its affective component. The bed nucleus of the stria terminalis (BNST) has been implicated in stress responses and negative affective states, such as anxiety, fear, and aversion. Recently, we demonstrated the crucial role of the BNST in the negative affective component of pain using the conditioned place aversion (CPA) test. In the present study, we investigated the involvement of the β -adrenoceptor–protein kinase A (PKA) signaling pathway within the BNST, in particular, within the ventral part of the BNST (vBNST), in pain-induced aversion in male Sprague Dawley rats. *In vivo* microdialysis showed that extracellular noradrenaline levels within the vBNST were significantly increased by intraplantar formalin injection. Using the CPA test, we found that intra-vBNST injection of timolol, a β -adrenoceptor antagonist, dose-dependently attenuated the intraplantar-formalin-induced CPA (F-CPA) without reducing nociceptive behaviors. Experiments with subtype-selective antagonists demonstrated the essential role of β_2 -adrenoceptors in F-CPA. Intra-vBNST injection of isoproterenol, a β -adrenoceptor agonist, dose-dependently produced CPA even in the absence of noxious stimulation. This isoproterenol-induced CPA was reversed by the coinjection of Rp-cyclic adenosine monophosphorothioate (Rp-cAMPS), a selective PKA inhibitor. Furthermore, intra-vBNST injection of Rp-cAMPS dose-dependently attenuated the F-CPA. Together, these results suggest that PKA activation within the vBNST via the enhancement of β -adrenergic transmission is important for the negative affective component of pain.

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Complementary Contributions of Prefrontal Neuron Classes in Abstract Numerical Categorization

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The primate prefrontal cortex (PFC) plays a cardinal role in forming abstract categories and concepts. However, it remains elusive how this is accomplished and to what extent the interaction of functionally distinct neuron classes underlies this representation. Here, we inferred the major cortical cell types, putative pyramidal cells, and interneurons by characterizing the waveforms of action potentials recorded in monkeys performing a cognitively demanding numerosity categorization task. Putative interneurons responded much faster than cells classified as pyramidal neurons and exhibited a higher reliability of category discrimination, whereas putative pyramidal cells showed a higher degree of category selectivity. An analysis of the numerosity tuning profiles and the temporal interactions of adjacent neurons indicated that inhibitory input by putative interneurons shapes the tuning to numerical categories of putative PFC pyramidal cells. These findings favor feedforward mechanisms subserving cognitive categorization and help to clarify cellular interactions in PFC microcircuits.

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Asymmetric Amplitude Modulations of Brain Oscillations Generate Slow Evoked Responses

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Electrophysiological data measured by electroencephalography and magnetoencephalography (MEG) are widely used to investigate human brain activity in various cognitive tasks. This is typically done by characterizing event-related potentials/fields or modulations of oscillatory activity (e.g., event-related synchronization) in response to cognitively relevant stimuli. Here, we provide a link between the two phenomena. An essential component of our theory is that peaks and troughs of oscillatory activity fluctuate asymmetrically; e.g., peaks are more strongly modulated than troughs in response to stimuli. As a consequence, oscillatory brain activity will not “average out” when multiple trials are averaged. Using MEG, we demonstrate that such asymmetric amplitude fluctuations of the oscillatory alpha rhythm explain the generation of

slow event-related fields. Furthermore, we provide a physiological explanation for the observed asymmetric amplitude fluctuations. In particular, slow event-related components are modulated by a wide range of cognitive tasks. Hence, our findings provide new insight into the physiological basis of cognitive modulation in event-related brain activity.

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Influence of Reward Delays on Responses of Dopamine Neurons

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Psychological and microeconomic studies have shown that outcome values are discounted by imposed delays. The effect, called temporal discounting, is demonstrated typically by choice preferences for sooner smaller rewards over later larger rewards. However, it is unclear whether temporal discounting occurs during the decision process when differently delayed reward outcomes are compared or during predictions of reward delays by pavlovian conditioned stimuli without choice. To address this issue, we investigated the temporal discounting behavior in a choice situation and studied the effects of reward delay on the value signals of dopamine neurons. The choice behavior confirmed hyperbolic discounting of reward value by delays on the order of seconds. Reward delay reduced the responses of dopamine neurons to pavlovian conditioned stimuli according to a hyperbolic decay function similar to that observed in choice behavior. Moreover, the stimulus responses increased with larger reward magnitudes, suggesting that both delay and magnitude constituted viable components of dopamine value signals. In contrast, dopamine responses to the reward itself increased with longer delays, possibly reflecting temporal uncertainty and partial learning. These dopamine reward value signals might serve as useful inputs for brain mechanisms involved in economic choices between delayed rewards.

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Amphetamine Activation of Hippocampal Drive of Mesolimbic Dopamine Neurons: A Mechanism of Behavioral Sensitization

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The repeated administration of psychostimulants induces an enhanced behavioral response to a subsequent drug challenge. This behavioral sensitization is proposed to model the increased drug craving observed in human psychostimulant abusers. Using *in vivo* extracellular recordings from identified ventral tegmental area dopamine (DA) neurons, we report that amphetamine-sensitized rats display an activation of ventral hippocampal neuron firing and a significantly greater number of spontaneously active DA neurons compared with saline-treated rats. Moreover, TTX inactivation of the ventral hippocampus restores DA neuron activity to control levels and also blocks the expression of locomotor sensitization. Taken as a whole, we propose that behavioral sensitization to psychostimulant drugs is attributable, at least in part, to persistent activation of the ventral hippocampus–nucleus accumbens pathway, with the resultant increase in tonic DA neuron firing enabling an abnormally higher response to subsequent psychostimulant administration.

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Sequence Reactivation in the Hippocampus Is Impaired in Aged Rats

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The hippocampus is thought to coordinate memory consolidation by reactivating traces from behavioral experience when the brain is not actively processing new input. In fact, during slow-wave sleep, the patterns of CA1 pyramidal cell ensemble activity correlations are reactivated in both young and aged rats. In addition to correlated activity patterns, repetitive track running also creates a recurring sequence of pyramidal cell activity. The present study compared CA1 sequence activity pattern replay in young and old animals during rest periods after behavior. Whereas the young rats exhibited significant sequence reactivation, it was markedly impaired in the aged animals. When the spatial memory scores of all animals were compared with the degree of sequence reactivation, there was a significant correlation. The novel finding that weak replay of temporal patterns has behavioral consequences, strengthens the idea that reactivation processes are integral to memory consolidation.

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Measures of Cortical Plasticity after Transcranial Paired Associative Stimulation Predict Changes in Electroencephalogram Slow-Wave Activity during Subsequent Sleep

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Sleep slow-wave activity (SWA) is thought to reflect sleep need, increasing in proportion to the previous time awake and decreasing during sleep, although the underlying mechanisms are unclear. Recent studies have shown that procedures presumably leading to local plastic changes in the cerebral cortex can lead to local changes in SWA during subsequent sleep. To further investigate the connection between cortical plasticity and sleep SWA, in this study we used a paired associative stimulation (PAS) protocol, in which median nerve stimuli were followed at different intervals (25 or 10 ms) by transcranial magnetic stimulation (TMS) pulses to the contralateral cortical hand area. As expected, such a protocol led to a sustained increase (long-term potentiation-like) or decrease (long-term depression-like) of cortical excitability as measured by motor evoked potentials. By using a TMS-compatible high-density electroencephalographic (EEG) system, we also found that, in individual subjects, TMS-evoked cortical responses over sensorimotor cortex changed with different interstimulus intervals. Moreover, during subsequent sleep, SWA increased locally in subjects whose TMS-evoked cortical responses had increased after PAS, and decreased in subjects whose cortical responses had decreased. Changes in TMS-evoked cortical EEG response and change in sleep SWA were localized to similar cortical regions and were positively correlated. Together, these results suggest that changes in cortical excitability in opposite directions lead to corresponding changes in local sleep regulation, as reflected by SWA, providing evidence for a tight relationship between cortical plasticity and sleep intensity.

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NEUROBIOLOGY OF DISEASE

Caveolin-1 Regulates Human Immunodeficiency Virus-1 Tat-Induced Alterations of Tight Junction Protein Expression via Modulation of the Ras Signaling

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The blood–brain barrier (BBB) is the critical structure for preventing human immunodeficiency virus (HIV) trafficking into the brain. Specific HIV proteins, such as Tat protein, can contribute to the dysfunction of tight junctions at the BBB and HIV entry into the brain. Tat is released by HIV-1-infected cells and can interact with a variety of cell surface receptors activating several signal transduction pathways, including those localized in caveolae. The present study focused on the mechanisms of Tat-induced caveolae-associated Ras signaling at the level of the BBB. Treatment with Tat activated the Ras pathway in human brain microvascular endothelial cells (HBMECs). However, caveolin-1 silencing markedly attenuated these effects. Because the integrity of the brain endothelium is regulated by intercellular tight junctions, these structural elements of the BBB were also evaluated in the present study. Exposure to Tat diminished the expression of several tight junction proteins, namely, occludin, zonula occludens (ZO)-1, and ZO-2 in the caveolar fraction of HBMECs. These effects were effectively protected by pharmacological inhibition of the Ras signaling and by silencing of caveolin-1. The present data indicate the importance of caveolae-associated signaling in the disruption of tight junctions on Tat exposure. They also demonstrate that caveolin-1 may constitute an early and critical modulator that controls signaling pathways leading to the disruption of tight junction proteins. Thus, caveolin-1 may provide an effective target to protect against Tat-induced HBMEC dysfunction and the disruption of the BBB in HIV-1-infected patients.

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Functional Convergence of Dopaminergic and Cholinergic Input Is Critical for Hippocampus-Dependent Working Memory

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Although Parkinson's disease is a movement disorder, in many patients cognitive dysfunction is an important clinical sign. It is not yet clear whether this is attributable solely to a decrease in dopamine levels, or whether other neurotransmitter systems might be involved as well. In the present study, the importance of the mesocorticolimbic dopamine pathway and a possible convergence with forebrain cholinergic projections to neocortex and hippocampus in the regulation of learning and memory abilities were investigated by using specific lesion paradigms in one or both systems. Lesioning of dopaminergic neurons in the ventral tegmental area resulted in an impaired performance in the reference memory task, whereas the execution of the working memory tasks appeared to be unaffected in the Morris water maze. Analysis of the swim paths revealed that the dopamine-depleted animals were capable of adapting a search strategy on a given testing day but failed to transfer this information to the next day, suggesting a deficit in information storage and/or recall. In contrast, cholinergic lesions alone were without effect in all test paradigms. However, when both dopamine and acetylcholine were depleted, animals were also impaired in the working memory task, indicating that a functional convergence of the inputs from these systems was critical for acquisition of spatial memory. Interestingly, such an additional acquisition deficit appeared only after hippocampal cholinergic depletion regardless of a concurrent

disruption of basolateral cholinergic afferents. Thus, further analyses of cholinergic alterations may prove useful in better understanding the cognitive symptoms in Parkinson's disease.

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Intra-Amygdaloid Injection of Kainic Acid in Rats with Genetic Absence Epilepsy: The Relationship of Typical Absence Epilepsy and Temporal Lobe Epilepsy

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We showed previously that genetic absence epilepsy rats from Strasbourg (GAERS) resist secondary generalization of focal limbic seizures after electrical kindling. We now investigate the effect of intra-amygdaloid injection of kainic acid, as another model of temporal lobe epilepsy, focusing on epileptogenesis, spike-and-wave discharges (SWDs), and the transition from basal to SWD states in GAERS. The EEG was recorded from the hippocampus and cortex of adult GAERS and Wistar rats before kainic acid injections into the basolateral amygdala and for 3 months thereafter. EEG and video recordings monitored SWDs and convulsive seizures. We analyzed spectral changes of the EEG during kainic acid-induced status epilepticus, SWDs, for 10 s before (silent period) and for 2 s before (transition period) SWDs. After the injection of kainic acid, all animals experienced convulsive seizures for at least 3 h. The first convulsive seizure was significantly delayed in GAERS compared with Wistar rats. SWDs and increases in power of the delta, alpha, and beta frequency ranges during the transition period disappeared after the kainic acid injection for 1–3 d and gradually reappeared. Power increases in the delta and alpha ranges were significantly correlated with the number of SWDs, in the beta and alpha ranges with their mean duration. Neo-Timm's staining at the end of experiments demonstrated that mossy fiber sprouting in GAERS is less pronounced than in Wistar rats. Our findings show that mechanisms underlying absence epilepsy and temporal lobe epilepsy interact with each other, although a site of this interaction remains to be defined.

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Ceramide Is Responsible for the Failure of Compensatory Nerve Sprouting in Apolipoprotein E Knock-Out Mice

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Apolipoprotein E (apoE) is a key transporter of the cholesterol and phospholipids required for membrane synthesis and nerve growth. We now report a virtual absence in apoE knock-out (KO) mice of normal nerve growth factor (NGF)-driven compensatory sprouting of undamaged cutaneous nociceptive nerves. In contrast, NGF-independent regeneration of crushed axons was unaffected. Essentially similar results came from aged wild-type mice. In apoE KO mice, the endogenous sprouting stimulus was suspect, because NGF administration induced normal sprouting; nevertheless, NGF increased normally in denervated skin, transported normally in the axons, and led to phosphorylation of trkA, erk1, and erk2. However, sprouting was restored in apoE KO mice (although not in aged mice) by fumonisin B1, an inhibitor of ceramide synthesis. A shotgun analysis revealed a wide array of changes in individual ceramide species in DRG neurons of apoE KO mice, and the changes for ceramide species OH_N15:0 made it a candidate inhibitor of sprouting (increased in apoE KO mice and normalized by fumonisin B1). Nevertheless, the unknown effects of individual ceramide species on sprouting, as well as the variability of their changed levels in apoE KO mice and how these were affected by fumonisin B1, support a different conclusion. We suggest that absence of apoE expression alters the balance among ceramide species to one that collectively inhibits compensatory sprouting, whereas fumonisin B1 establishes a new balance that allows sprouting. Nontoxic ceramide modulators might usefully promote sprouting and circuitry repair in neurodegenerative disorders in which ceramide species are perturbed, adding to the benefits of reducing ceramide-induced neuronal apoptosis.

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