

This Week in The Journal

● Cellular/Molecular

5-HT Directly Enhances ASIC3 Currents

Xiang Wang, Wei-Guang Li, Ye Yu, Xian Xiao, Jin Cheng, et al.

(see pages 4265–4279)

Tissue damage causes gradual acidosis and release of inflammatory mediators, both of which enhance pain responses. Acidosis depolarizes somatosensory neurons by opening acid-sensing ion channels (ASICs), particularly ASIC3. ASIC3-mediated currents are biphasic, consisting of a large transient current and a smaller, sustained response. The latter is likely to play a larger role during injury-induced acidosis, because the former desensitizes during gradual acidosis. Several inflammatory mediators increase ASIC expression, thus enhancing the effect of acidosis after tissue damage. Wang et al. have discovered that one of these inflammatory mediators, 5-HT, also enhances sustained ASIC currents via direct interaction with the channel. In mouse dorsal root ganglion neurons, 5-HT enhanced currents induced by gradual acidosis independently of classical 5-HT receptors and intracellular signaling cascades, likely by binding to the “nonproton ligand sensing” domain of ASIC3. Thus, 5-HT increased the number of action potentials induced by acidic solution. Additionally, injecting 5-HT along with mild acid into mouse paws prolonged the nocifensive licking response.

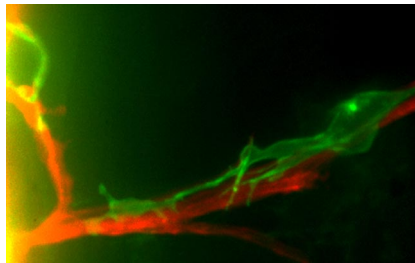
● Development/Plasticity/Repair

Notch Allows Perineurial Cells to Exit the CNS

Laura A. Binari, Gwendolyn M. Lewis, and Sarah Kucenas

(see pages 4241–4252)

Motor nerves are encased by a continuous, protective sheath, the perineurium, composed of perineurial glial cells connected by tight junctions. Perineurial cells are generated in the CNS and migrate through motor axon exit points (MEPs) immediately following motor axon growth cones. Binari et al. found that, in



Perineurial glial cells (green) exit the CNS (left) and migrate along motor root axons (red). See the article by Binari et al. for details.

zebrafish larvae, perineurial glial cells formed a continuous chain as they exited the CNS and migrated along motor nerves. Upon leaving the CNS, perineurial cells began to express Notch, which was downregulated when migration was finished. Blocking Notch signaling before perineurial glia exited the CNS prevented their exit: although leader cells extended filopodia through MEPs, their somata remained within the CNS, even after Notch signaling was restored. Blocking Notch signaling after perineurial cells exited the CNS did not impair migration, but it did inhibit the formation of the tight junctions between cells that characterize the mature perineurium. Fewer Schwann cells were present along peripheral nerves when Notch signaling was disrupted, suggesting that perineurial cells influence myelination.

● Systems/Circuits

Swim CPG Drives Compensatory Eye Movements in Frogs

Géraldine von Uckermann, Didier Le Ray, Denis Combes, Hans Straka, and John Simmers

(see pages 4253–4264)

To maintain a stable image of the world during locomotion, animals move their eyes to compensate for self-generated movement. In terrestrial vertebrates, the head moves in three dimensions, and feedback from the vestibular system is thought to be essential for directing eye movements. But in swimming *Xenopus* tadpoles, head movements are restricted to the horizontal plane, and conjugate left–right rotation of the eyes, driven by

input from the central pattern generator (CPG) that drives swimming, is sufficient to compensate for head movements. After metamorphosis, however, frogs swim by synchronously kicking their hindlegs, resulting in linear forward acceleration. von Uckermann et al. observed that both eyes rotated inward, non-conjugately, during forward movement. Correspondingly, medial rectus motor neurons, which rotate the eyes inward, were synchronously active in phase with hindlimb extensor motor neurons. This occurred in isolated brainstem/spinal cord preparations, in the absence of visual or vestibular feedback, suggesting that input from the swim CPG continues to instruct compensatory eye movements after metamorphosis.

● Neurobiology of Disease

Microglia Phagocytose Cortical Neuron Precursors

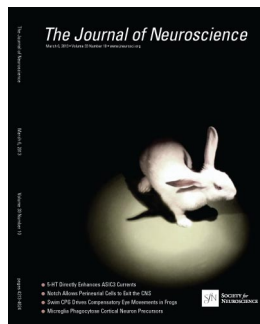
Christopher L. Cunningham, Verónica Martínez-Cerdeño, and Stephen C. Noctor

(see pages 4216–4233)

Early in cortical development, neural precursor cells (NPCs) proliferate, generating a large pool of progenitors in the subventricular zone (SVZ). As development proceeds, the progenitor pool is exhausted, preventing excessive growth of the brain. The reduction of the progenitor pool is thought to result from a combination of symmetric cell divisions that produce two postmitotic neurons and NPC apoptosis. But Cunningham et al. suggest that microglia also limit the size of the progenitor pool. The number of microglia in developing rat and primate cortex increased as corticogenesis proceeded. Microglia were concentrated in two bands in the SVZ, where they contacted and engulfed NPCs. Notably, most of the targeted NPCs were mitotically active, and few appeared to be undergoing apoptosis. After corticogenesis was complete, microglia became evenly distributed throughout the cortex. Finally, increasing the proportion of M1-type microglia reduced NPC numbers, whereas increasing the proportion of M2-type microglia or selectively killing microglia *in utero* increased the number of NPCs and immature neurons.

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Cover legend: The rostromedial prefrontal cortex in rabbits potently inhibits acquired eyelid responses until the need for the acquired response is fully confirmed. In this regard, the rostromedial prefrontal cortex seems to act as flip-flop mechanism controlling the expression of reflex and acquired behaviors depending on the environmental circumstances. Photography by J.A. Santos and J.C. López-Ramos; editing by R. Leal-Campanario. For more information, see the article by Leal-Campanario in this issue (pages 4378 – 4386).

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4623 **Correction:** The article “Neuroestrogens Rapidly Regulate Sexual Motivation But Not Performance” by Aurore L. Seredynski, Jacques Balthazart, Virginie J. Christophe, Gregory F. Ball, and Charlotte A. Cornil appeared on pages 164–174 of the January 2, 2013 issue. A correction for that article appears on page 4623.

Correction: The article “Glutamate Receptor 1 Phosphorylation at Serine 831 and 845 Modulates Seizure Susceptibility and Hippocampal Hyperexcitability after Early Life Seizures” by Sanjay N. Rakhade, Erin F. Fitzgerald, Peter M. Klein, Chengwen Zhou, Hongyu Sun, Richard L. Hanganir, and Frances E. Jensen appeared on pages 17800–17812 of the December 5, 2012 issue. A correction for that article appears on page 4623.

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Inhibition of a SNARE-Sensitive Pathway in Astrocytes Attenuates Damage following Stroke

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A strong body of research has defined the role of excitotoxic glutamate in animal models of brain ischemia and stroke; however, clinical trials of glutamate receptor antagonists have demonstrated their limited capacity to prevent brain damage following ischemia. We propose that astrocyte–neuron signaling represents an important modulatory target that may be useful in mediating damage following stroke. To assess the impact of astrocyte signaling on damage following stroke, we have used the astrocyte-specific dominant-negative SNARE mouse model (dnSNARE). Recent findings have shown that the astrocytic SNARE signaling pathway can affect neuronal excitability by regulating the surface expression of NMDA receptors. Using focal photothrombosis via the Rose Bengal method, as well as excitotoxic NMDA lesions, we show that dnSNARE animals exhibited a sparing of damaged tissue quantified using Nissl and NeuN staining. At the same time point, animals were also tested in behavioral tasks that probe the functional integrity of stroke- or lesion-damaged motor and somatosensory areas. We found that dnSNARE mice performed significantly better than littermate controls on rung walk and adhesive dot removal tasks following lesion. Together, our results demonstrate the important role of astrocytic signaling under ischemic conditions. Drugs targeting astrocyte signaling have a potential benefit for the outcome of stroke in human patients by limiting the spread of damage.

The Journal of Neuroscience, March 6, 2013 • 33(10):4234–4240

The Mental Cost of Cognitive Enhancement

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Noninvasive brain stimulation provides a potential tool for affecting brain functions in the typical and atypical brain and offers in several cases an alternative to pharmaceutical intervention. Some studies have suggested that transcranial electrical stimulation (TES), a form of noninvasive brain stimulation, can also be used to enhance cognitive performance. Critically, research so far has primarily focused on optimizing protocols for effective stimulation, or assessing potential physical side effects of TES while neglecting the possibility of cognitive side effects. We assessed this possibility by targeting the high-level cognitive abilities of learning and automaticity in the mathematical domain. Notably, learning and automaticity represent critical abilities for potential cognitive enhancement in typical and atypical populations. Over 6 d, healthy human adults underwent cognitive training on a new numerical notation while receiving TES to the posterior parietal cortex or the dorsolateral prefrontal cortex. Stimulation to the posterior parietal cortex facilitated numerical learning, whereas automaticity for the learned material was impaired. In contrast, stimulation to the dorsolateral prefrontal cortex impaired the learning process, whereas automaticity for the learned material was enhanced. The observed double dissociation indicates that cognitive enhancement through TES can occur at the expense of other cognitive functions. These findings have important implications for the future use of enhancement technologies for neurointervention and performance improvement in healthy populations.

The Journal of Neuroscience, March 6, 2013 • 33(10):4482–4486

Effect of Limb Lengthening on Internodal Length and Conduction Velocity of Peripheral Nerve

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The influences of axon diameter, myelin thickness, and internodal length on the velocity of conduction of peripheral nerve action potentials are unclear. Previous studies have demonstrated a strong dependence of conduction velocity on internodal length. However, a theoretical analysis has suggested that this relationship may be lost above a nodal separation of ~0.6 mm. Here we measured nerve conduction velocities in a rabbit model of limb lengthening that produced compensatory increases in peripheral nerve growth. Divided tibial bones in one hindlimb were gradually lengthened at 0.7 mm per day using an external frame attached to the bone. This was associated with a significant increase (33%) of internodal length (0.95–1.3 mm) in axons of the tibial nerve that varied in proportion to the mechanical strain in the nerve of the lengthened limb. Axonal diameter, myelin thickness, and g-ratios were not significantly altered by limb lengthening. Despite the substantial increase in internodal length, no significant change was detected in conduction velocity (~43 m/s) measured either *in vivo* or in isolated tibial nerves. The results demonstrate that the internode remains plastic in the adult but that increases in internodal length of myelinated adult nerve axons do not result in either deficiency or proportionate increases in their conduction velocity and support the view that the internodal lengths of nerves reach a plateau beyond which their conduction velocities are no longer sensitive to increases in internodal length.

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Serotonin Facilitates Peripheral Pain Sensitivity in a Manner That Depends on the Nonproton Ligand Sensing Domain of ASIC3 Channel

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Tissue acidosis and inflammatory mediators play critical roles in inflammatory pain. Extracellular acidosis activates acid-sensing ion channels (ASICs), which have emerged as key sensors for extracellular protons in the central and peripheral nervous systems and play key roles in pain sensation and transmission. Additionally, inflammatory mediators, such as serotonin (5-HT), are known to enhance pain sensation. However, functional interactions among protons, inflammatory mediators, and ASICs in pain sensation are poorly understood. In the present study, we show that 5-HT, a classical pro-inflammatory mediator, specifically enhances the proton-evoked sustained, but not transient, currents mediated by homomeric ASIC3 channels and heteromeric ASIC3/1a and ASIC3/1b channels. Unexpectedly, the effect of 5-HT on ASIC3 channels does not involve activation of 5-HT receptors, but is mediated via a functional interaction between 5-HT and ASIC3 channels. We further show that the effect of 5-HT on ASIC3 channels depends on the newly identified nonproton ligand sensing domain. Finally, coapplication of 5-HT and acid significantly increased pain-related behaviors as assayed by the paw-licking test in mice, which was largely attenuated in ASIC3 knock-out mice, and inhibited by the nonselective ASIC inhibitor amiloride. Together, these data identify ASIC3 channels as an unexpected molecular target for acute actions of 5-HT in inflammatory pain sensation and reveal an important role of ASIC3 channels in regulating inflammatory pain via coincident detection of extracellular protons and inflammatory mediators.

The Journal of Neuroscience, March 6, 2013 • 33(10):4265–4279

Membrane Trafficking of NADPH Oxidase p47^{phox} in Paraventricular Hypothalamic Neurons Parallels Local Free Radical Production in Angiotensin II Slow-Pressor Hypertension

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NADPH oxidase-generated reactive oxygen species (ROS) are highly implicated in the development of angiotensin II (AngII)-dependent hypertension mediated in part through the hypothalamic paraventricular nucleus (PVN). This region contains vasopressin and non-vasopressin neurons that are responsive to cardiovascular dysregulation, but it is not known whether ROS is generated by one or both cell types in response to “slow-pressor” infusion of AngII. We addressed this question using ROS imaging and electron microscopic dual labeling for vasopressin and p47^{phox}, a cytoplasmic NADPH oxidase subunit requiring mobilization to membranes for the initiation of ROS production. C57BL/6 mice or vasopressin-enhanced green fluorescent protein (VP-eGFP) mice were infused systemically with saline or AngII (600 ng · kg⁻¹ · min⁻¹, s.c.) for 2 weeks, during which they slowly developed hypertension. Ultrastructural analysis of the PVN demonstrated p47^{phox} immunolabeling in many glial and neuronal profiles, most of which were postsynaptic dendrites. Compared with saline, AngII recipient mice had a significant increase in p47^{phox} immunolabeling on endomembranes just beneath the plasmalemmal surface (+42.1 ± 11.3%; *p* < 0.05) in non-vasopressin dendrites. In contrast, AngII infusion decreased p47^{phox} immunolabeling on the plasma membrane (−35.5 ± 16.5%; *p* < 0.05) in vasopressin dendrites. Isolated non-VP-eGFP neurons from the PVN of AngII-infused mice also showed an increase in baseline ROS production not seen in VP-eGFP neurons. Our results suggest that chronic low-dose AngII may offset the homeostatic control of blood pressure by differentially affecting membrane assembly of NADPH oxidase and ROS production in vasopressin and non-vasopressin neurons located within the PVN.

The Journal of Neuroscience, March 6, 2013 • 33(10):4308–4316

Calpain-2-Mediated PTEN Degradation Contributes to BDNF-Induced Stimulation of Dendritic Protein Synthesis

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Memory consolidation has been suggested to be protein synthesis dependent. Previous data indicate that BDNF-induced dendritic protein synthesis is a key event in memory formation through activation of the mammalian target of rapamycin (mTOR) pathway. BDNF also activates calpain, a calcium-dependent cysteine protease, which has been shown to play a critical role in learning and memory. This study was therefore directed at testing the hypothesis that calpain activity is required for BDNF-stimulated local protein synthesis, and at identifying the underlying molecular mechanism. In rat hippocampal slices, cortical synaptoneurosome, and cultured neurons,

BDNF-induced mTOR pathway activation and protein translation were blocked by calpain inhibition. BDNF treatment rapidly reduced levels of hamartin and tuberlin, negative regulators of mTOR, in a calpain-dependent manner. Treatment of brain homogenates with purified calpain-1 and calpain-2 truncated both proteins. BDNF treatment increased phosphorylation of both Akt and ERK, but only the effect on Akt was blocked by calpain inhibition. Levels of phosphatase and tensin homolog deleted on chromosome 10 (PTEN), a phosphatase that inactivates Akt, were decreased following BDNF treatment, and calpain inhibition reversed this effect. Calpain-2, but not calpain-1, treatment of brain homogenates resulted in PTEN degradation. In cultured cortical neurons, knockdown of calpain-2, but not calpain-1, by small interfering RNA completely suppressed the effect of BDNF on mTOR activation. Our results reveal a critical role for calpain-2 in BDNF-induced mTOR signaling and dendritic protein synthesis via PTEN, hamartin, and tuberlin degradation. This mechanism therefore provides a link between proteolysis and protein synthesis that might contribute to synaptic plasticity.

The Journal of Neuroscience, March 6, 2013 • 33(10):4317–4328

Differential Control of Opioid Antinociception to Thermal Stimuli in a Knock-In Mouse Expressing Regulator of G-Protein Signaling-Insensitive $G\alpha_o$ Protein

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Regulator of G-protein signaling (RGS) proteins classically function as negative modulators of G-protein-coupled receptor signaling. *In vitro*, RGS proteins have been shown to inhibit signaling by agonists at the μ -opioid receptor, including morphine. The goal of the present study was to evaluate the contribution of endogenous RGS proteins to the antinociceptive effects of morphine and other opioid agonists. To do this, a knock-in mouse that expresses an RGS-insensitive (RGSi) mutant $G\alpha_o$ protein, $G\alpha_o^{G184S}$ ($G\alpha_o$ RGSi), was evaluated for morphine or methadone antinociception in response to noxious thermal stimuli. Mice expressing $G\alpha_o$ RGSi subunits exhibited a naltrhexone-sensitive enhancement of baseline latency in both the hot-plate and warm-water tail-withdrawal tests. In the hot-plate test, a measure of supraspinal nociception, morphine antinociception was increased, and this was associated with an increased ability of opioids to inhibit presynaptic GABA neurotransmission in the periaqueductal gray. In contrast, antinociception produced by either morphine or methadone was reduced in the tail-withdrawal test, a measure of spinal nociception. In whole-brain and spinal cord homogenates from mice expressing $G\alpha_o$ RGSi subunits, there was a small loss of $G\alpha_o$ expression and an accompanying decrease in basal G-protein activity. Our results strongly support a role for RGS proteins as negative regulators of opioid supraspinal antinociception and also reveal a potential novel function of RGS proteins as positive regulators of opioid spinal antinociceptive pathways.

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Noddy, a Mouse Harboring a Missense Mutation in Protocadherin-15, Reveals the Impact of Disrupting a Critical Interaction Site between Tip-Link Cadherins in Inner Ear Hair Cells

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In hair cells of the inner ear, sound or head movement increases tension in fine filaments termed tip links, which in turn convey force to mechanosensitive ion channels to open them. Tip links are formed by a tetramer of two cadherin proteins: protocadherin 15 (PCDH15) and cadherin 23 (CDH23), which have 11 and 27 extracellular cadherin (EC) repeats, respectively. Mutations in either protein cause inner ear disorders in mice and humans. We showed recently that these two cadherins bind tip-to-tip in a “handshake” mode that involves the EC1 and EC2 repeats of both proteins. However, a paucity of appropriate animal models has slowed our understanding both of the interaction and of how mutations of residues within the predicted interface compromise tip link integrity. Here, we present *noddy*, a new mouse model for hereditary deafness. Identified in a forward genetic screen, *noddy* homozygotes lack inner ear function. Mapping and sequencing showed that *noddy* mutant mice harbor an isoleucine-to-asparagine (I108N) mutation in the EC1 repeat of PCDH15. Residue I108 interacts with CDH23 EC2 in the handshake and its mutation impairs the interaction *in vitro*. The *noddy* mutation allowed us to determine the consequences of blocking the handshake *in vivo*: tip link formation and bundle morphology are disrupted, and mechanotransduction channels fail to remain open at rest. These results offer new insights into the interaction between PCDH15 and CDH23 and help explain the etiology of human deafness linked to mutations in the tip-link interface.

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Functional Mechanotransduction Is Required for Cisplatin-Induced Hair Cell Death in the Zebrafish Lateral Line

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Cisplatin, one of the most commonly used anticancer drugs, is known to cause inner ear hair cell damage and hearing loss. Despite much investigation into mechanisms of cisplatin-induced hair cell death, little is known about the mechanism whereby cisplatin is selectively toxic to hair cells. Using hair cells of the zebrafish lateral line, we found that chemical inhibition of mechanotransduction with quinine and EGTA protected against cisplatin-induced hair cell death. Furthermore, we found that the zebrafish mutants *mariner* (*myo7aa*) and *sputnik* (*cad23*) that lack functional mechanotransduction were resistant to cisplatin-induced hair cell death. Using a fluorescent analog of cisplatin, we found that chemical or genetic inhibition of mechanotransduction prevented its uptake. These findings demonstrate that cisplatin-induced hair cell death is dependent on functional mechanotransduction in the zebrafish lateral line.

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Lack of Evidence for Vesicular Glutamate Transporter Expression in Mouse Astrocytes

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The concept of a tripartite synapse including a presynaptic terminal, a postsynaptic spine, and an astrocytic process that responds to neuronal activity by fast gliotransmitter release, confers to the electrically silent astrocytes an active role in information processing. However, the mechanisms of gliotransmitter release are still highly controversial. The reported expression of all three vesicular glutamate transporters (VGLUT1–3) by astrocytes suggests that astrocytes, like neurons, may release glutamate by exocytosis. However, the demonstration of astrocytic VGLUT expression is largely based on immunostaining, and the possibility of nonspecific labeling needs to be systematically addressed. We therefore examined the expression of VGLUT1–3 in astrocytes, both in culture and *in situ*. We used Western blots and single-vesicle imaging by total internal reflection fluorescence microscopy in live cultured astrocytes, and confocal microscopy, at the cellular level in cortical, hippocampal, and cerebellar brain slices, combined with quantitative image analysis. Control experiments were systematically performed in cultured astrocytes using wild-type, VGLUT1–3 knock-out, VGLUT1^{Venus} knock-in, and VGLUT2-EGFP transgenic mice. In fixed brain slices, we quantified the degree of overlap between VGLUT1–3 and neuronal or astrocytic markers, both in an object-based manner using fluorescence line profiles, and in a pixel-based manner using dual-color scatter plots followed by the calculation of Pearson's correlation coefficient over all pixels with intensities significantly different from background. Our data provide no evidence in favor of the expression of any of the three VGLUTs by gray matter protoplasmic astrocytes of the primary somatosensory cortex, the thalamic ventrobasal nucleus, the hippocampus, and the cerebellum.

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Disruption of the Presynaptic Cytomatrix Protein Bassoon Degrades Ribbon Anchorage, Multiquantal Release, and Sound Encoding at the Hair Cell Afferent Synapse

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Inner hair cells (IHCs) of the cochlea use ribbon synapses to transmit auditory information faithfully to spiral ganglion neurons (SGNs). In the present study, we used genetic disruption of the presynaptic scaffold protein bassoon in mice to manipulate the morphology and function of the IHC synapse. Although partial-deletion mutants lacking functional bassoon ($Bsn^{\Delta Ex4/5}$) had a near-complete loss of ribbons from the synapses (up to 88% ribbonless synapses), gene-trap mutants (Bsn^{gt}) showed weak residual expression of bassoon and 56% ribbonless synapses, whereas the remaining 44% had a loosely anchored ribbon. Patch-clamp recordings and synaptic $Ca_v1.3$ immunolabeling indicated a larger number of Ca^{2+} channels for Bsn^{gt} IHCs compared with $Bsn^{\Delta Ex4/5}$ IHCs and for Bsn^{gt} ribbon-occupied versus Bsn^{gt} ribbonless synapses. An intermediate phenotype of Bsn^{gt} IHCs was also found by membrane capacitance measurements for sustained exocytosis, but not for the size of the readily releasable vesicle pool. The frequency and amplitude of EPSCs were reduced in $Bsn^{\Delta Ex4/5}$ mouse SGNs, whereas their postsynaptic AMPA receptor clusters were largely unaltered. Sound coding in SGN, assessed by recordings of single auditory nerve fibers and their population responses *in vivo*, was similarly affected in Bsn^{gt} and $Bsn^{\Delta Ex4/5}$ mice. Both genotypes showed impaired sound onset coding and reduced evoked and spontaneous spike rates. In summary, reduced bassoon expression or complete lack

of full-length bassoon impaired sound encoding to a similar extent, which is consistent with the comparable reduction of the readily releasable vesicle pool. This suggests that the remaining loosely anchored ribbons in Bsn^{8f} IHCs were functionally inadequate or that ribbon independent mechanisms dominated the coding deficit.
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Stabilization of Actin Bundles by a Dynamin 1/Cortactin Ring Complex Is Necessary for Growth Cone Filopodia

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Dynamin GTPase, a key molecule in endocytosis, mechanically severs the invaginated membrane upon GTP hydrolysis. Dynamin functions also in regulating actin cytoskeleton, but the mechanisms are yet to be defined. Here we show that dynamin 1, a neuronal isoform of dynamin, and cortactin form ring complexes, which twine around F-actin bundles and stabilize them. By negative-staining EM, dynamin 1–cortactin complexes appeared as “open” or “closed” rings depending on guanine nucleotide conditions. By pyrene actin assembly assay, dynamin 1 stimulated actin assembly in mouse brain cytosol. *In vitro* incubation of F-actin with both dynamin 1 and cortactin led to the formation of long and thick actin bundles, on which dynamin 1 and cortactin were periodically colocalized in puncta. A depolymerization assay revealed that dynamin 1 and cortactin increased the stability of actin bundles, most prominently in the presence of GTP. In rat cortical neurons and human neuroblastoma cell line, SH-SY5Y, both dynamin 1 and cortactin localized on actin filaments and the bundles at growth cone filopodia as revealed by immunoelectron microscopy. In SH-SY5Y cell, acute inhibition of dynamin 1 by application of dynamin inhibitor led to growth cone collapse. Cortactin knockdown also reduced growth cone filopodia. Together, our results strongly suggest that dynamin 1 and cortactin ring complex mechanically stabilizes F-actin bundles in growth cone filopodia. Thus, the GTPase-dependent mechanochemical enzyme property of dynamin is commonly used both in endocytosis and regulation of F-actin bundles by a dynamin 1–cortactin complex.

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Precise Neural Stimulation in the Retina Using Focused Ultrasound

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Focused ultrasound is a promising noninvasive technology for neural stimulation. Here we use the isolated salamander retina to characterize the effect of ultrasound on an intact neural circuit and compared these effects with those of visual stimulation of the same retinal ganglion cells. Ultrasound stimuli at an acoustic frequency of 43 MHz and a focal spot diameter of 90 μ m delivered from a piezoelectric transducer evoked stable responses with a temporal precision equal to strong visual responses but with shorter latency. By presenting ultrasound and visual stimulation together, we found that ultrasonic stimulation rapidly modulated visual sensitivity but did not change visual temporal filtering. By combining pharmacology with ultrasound stimulation, we found that ultrasound did not directly activate retinal ganglion cells but did in part activate interneurons beyond photoreceptors. These results suggest that, under conditions of strong localized stimulation, timing variability is largely influenced by cells beyond photoreceptors. We conclude that ultrasonic stimulation is an effective and spatiotemporally precise method to activate the retina. Because the retina is the most accessible part of the CNS *in vivo*, ultrasonic stimulation may have diagnostic potential to probe remaining retinal function in cases of photoreceptor degeneration, and therapeutic potential for use in a retinal prosthesis. In addition, because of its noninvasive properties and spatiotemporal resolution, ultrasound neurostimulation promises to be a useful tool to understand dynamic activity in pharmacologically defined neural pathways in the retina.

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Pituitary Adenylate Cyclase 1 Receptor Internalization and Endosomal Signaling Mediate the Pituitary Adenylate Cyclase Activating Polypeptide-Induced Increase in Guinea Pig Cardiac Neuron Excitability

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After G-protein-coupled receptor activation and signaling at the plasma membrane, the receptor complex is often rapidly internalized via endocytic vesicles for trafficking into various intracellular compartments and pathways. The formation of signaling endosomes is recognized as a mechanism that produces sustained intracellular signals that may be distinct from those generated at the cell surface for cellular responses including growth, differentiation, and survival. Pituitary adenylate cyclase activating polypeptide (PACAP; *Adcyap1*) is a potent neurotransmitter/neurotrophic peptide and mediates its diverse cellular functions in part through internalization of its cognate G-protein-coupled PAC1 receptor (PAC1R; *Adcyap1r1*). In the present study, we examined whether PAC1R endocytosis participates in the regulation of neuronal excitability. Although PACAP increased excitability in 90% of guinea pig cardiac neurons, pretreatment with Pitstop 2 or dynasore to inhibit clathrin and dynamin I/II, respectively, suppressed the PACAP effect. Subsequent addition of inhibitor after the PACAP-induced increase in excitability developed gradually attenuated excitability

with no changes in action potential properties. Likewise, the PACAP-induced increase in excitability was markedly decreased at ambient temperature. Receptor trafficking studies with GFP-PAC1 cell lines demonstrated the efficacy of Pitstop 2, dynasore, and low temperatures at suppressing PAC1R endocytosis. In contrast, brefeldin A pretreatments to disrupt Golgi vesicle trafficking did not blunt the PACAP effect, and PACAP/PAC1R signaling still increased neuronal cAMP production even with endocytic blockade. Our results demonstrate that PACAP/PAC1R complex endocytosis is a key step for the PACAP modulation of cardiac neuron excitability.

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DEVELOPMENT/PLASTICITY/REPAIR

Perineurial Glia Require Notch Signaling during Motor Nerve Development but Not Regeneration

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Motor nerves play the critical role of shunting information out of the CNS to targets in the periphery. Their formation requires the coordinated development of distinct cellular components, including motor axons and the Schwann cells and perineurial glia that ensheath them. During nervous system assembly, these glial cells must migrate long distances and terminally differentiate, ensuring the efficient propagation of action potentials. Although we know quite a bit about the mechanisms that control Schwann cell development during this process, nothing is known about the mechanisms that mediate the migration and differentiation of perineurial glia. Using *in vivo* imaging in zebrafish, we demonstrate that Notch signaling is required for both perineurial migration and differentiation during nerve formation, but not regeneration. Interestingly, loss of Notch signaling in perineurial cells also causes a failure of Schwann cell differentiation, demonstrating that Schwann cells require perineurial glia for aspects of their own development. These studies describe a novel mechanism that mediates multiple aspects of perineurial development and reveal the critical importance of perineurial glia for Schwann cell maturation and nerve formation.

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Endocannabinoid-Dependent Long-Term Depression in a Nociceptive Synapse Requires Coordinated Presynaptic and Postsynaptic Transcription and Translation

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Endocannabinoids (eCBs) play an important role in long-term regulation of synaptic signaling in both vertebrates and invertebrates. In this study, the role of transcription- and translation-dependent processes in presynaptic versus postsynaptic neurons was examined during eCB-mediated synaptic plasticity in the CNS of the leech. Low-frequency stimulation (LFS) of non-nociceptive afferents elicits eCB-dependent long-term depression (eCB-LTD) heterosynaptically in nociceptive synapses that lasts at least 2 h. Bath application of emetine, a protein synthesis inhibitor, blocked eCB-LTD after afferent LFS or exogenous eCB application, indicating that this depression was translation dependent. Bath application of actinomycin D, an irreversible RNA synthesis inhibitor, or 5,6-dichlorobenzimidazole 1- β -D-ribofuranoside (DRB), a reversible RNA synthesis inhibitor, also prevented eCB-LTD. Selective injection of DRB or emetine into the presynaptic or postsynaptic neuron before LFS indicated that eCB-LTD required transcription and translation in the postsynaptic neuron but only translation in the presynaptic cell. Depression observed immediately after LFS was also blocked when these transcription- and translation-dependent processes were inhibited. It is proposed that induction of eCB-LTD in this nociceptive synapse requires the coordination of presynaptic protein synthesis and postsynaptic mRNA and protein synthesis. These findings provide significant insights into both eCB-based synaptic plasticity and understanding how activity in non-nociceptive afferents modulates nociceptive pathways.

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Plasticity of Hypothalamic Dopamine Neurons during Lactation Results in Dissociation of Electrical Activity and Release

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Tuberoinfundibular dopamine (TIDA) neurons are the central regulators of prolactin (PRL) secretion. Their extensive functional plasticity allows a change from low PRL secretion in the non-pregnant state to the condition of hyperprolactinemia that characterizes lactation. To allow this rise in PRL, TIDA neurons are thought to become unresponsive to PRL at lactation and functionally silenced. Here we show that, contrary to expectations, the electrical properties of the system were not modified during

lactation and that the neurons remained electrically responsive to a PRL stimulus, with PRL inducing an acute increase in their firing rate during lactation that was identical to that seen in non-pregnant mice. Furthermore, we show a long-term organization of TIDA neuron electrical activity with an harmonization of their firing rates, which remains intact during lactation. However, PRL-induced secretion of dopamine (DA) at the median eminence was strongly blunted during lactation, at least in part attributable to lack of phosphorylation of tyrosine hydroxylase, the key enzyme involved in DA synthesis. We therefore conclude that lactation, rather than involving electrical silencing of TIDA neurons, represents a condition of decoupling between electrical activity at the cell body and DA secretion at the median eminence.

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SPARC Regulates Microgliosis and Functional Recovery following Cortical Ischemia

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Secreted protein acidic rich in cysteine (SPARC) is a matricellular protein that modulates the activity of growth factors, cytokines, and extracellular matrix to play multiple roles in tissue development and repair, such as cellular adhesion, migration, and proliferation. Throughout the CNS, SPARC is highly localized in mature ramified microglia, but its role in microglia—in development or during response to disease or injury—is not understood. In the postnatal brain, immature amoeboid myeloid precursors only induce SPARC expression after they cease proliferation and migration, and transform into mature, ramified resting microglia. SPARC null/CX3CR1-GFP reporter mice reveal that SPARC regulates the distribution and branching of mature microglia, with significant differences between cortical gray and white matter in both controls and SPARC nulls. Following ischemic and excitotoxic lesion, reactive, hypertrophic microglia rapidly downregulate and release SPARC at the lesion, concomitant with reactive, hypertrophic perilesion astrocytes upregulating SPARC. After photothrombotic stroke in the forelimb sensorimotor cortex, SPARC nulls demonstrate enhanced microgliosis in and around the lesion site, which accompanies significantly enhanced functional recovery by 32 d after lesion. Microglia from SPARC nulls also intrinsically proliferate at a greater rate *in vitro*—an enhanced effect that can be rescued by the addition of exogenous SPARC. SPARC is thus a novel regulator of microglial proliferation and structure, and, in addition to regulating glioma progression, may play an important role in differently regulating the gray and white matter microglial responses to CNS lesion—and modulating behavioral recovery—after injury.

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A Sympathetic Neuron Autonomous Role for Egr3-Mediated Gene Regulation in Dendrite Morphogenesis and Target Tissue Innervation

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Egr3 is a nerve growth factor (NGF)-induced transcriptional regulator that is essential for normal sympathetic nervous system development. Mice lacking Egr3 in the germline have sympathetic target tissue innervation abnormalities and physiologic sympathetic dysfunction similar to humans with dysautonomia. However, since Egr3 is widely expressed and has pleiotropic function, it has not been clear whether it has a role within sympathetic neurons and if so, what target genes it regulates to facilitate target tissue innervation. Here, we show that Egr3 expression within sympathetic neurons is required for their normal innervation since isolated sympathetic neurons lacking Egr3 have neurite outgrowth abnormalities when treated with NGF and mice with sympathetic neuron-restricted Egr3 ablation have target tissue innervation abnormalities similar to mice lacking Egr3 in all tissues. Microarray analysis performed on sympathetic neurons identified many target genes deregulated in the absence of Egr3, with some of the most significantly deregulated genes having roles in axonogenesis, dendritogenesis, and axon guidance. Using a novel genetic technique to visualize axons and dendrites in a subpopulation of randomly labeled sympathetic neurons, we found that Egr3 has an essential role in regulating sympathetic neuron dendrite morphology and terminal axon branching, but not in regulating sympathetic axon guidance to their targets. Together, these results indicate that Egr3 has a sympathetic neuron autonomous role in sympathetic nervous system development that involves modulating downstream target genes affecting the outgrowth and branching of sympathetic neuron dendrites and axons.

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SYSTEMS/CIRCUITS

Spinal Efference Copy Signaling and Gaze Stabilization during Locomotion in Juvenile *Xenopus* Frogs

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In swimming *Xenopus laevis* tadpoles, gaze stabilization is achieved by efference copies of spinal locomotory CPG output that produce rhythmic extraocular motor activity appropriate for minimizing motion-derived visual disturbances. During metamorphosis, *Xenopus* switches its locomotory mechanism from larval tail-based undulatory movements to bilaterally synchronous hindlimb kick propulsion in the adult. The change in locomotory mode leads to body motion dynamics that no longer require

conjugate left–right eye rotations for effective retinal image stabilization. Using *in vivo* kinematic analyses, *in vitro* electrophysiological recordings and specific CNS lesions, we have investigated spino-extraocular motor coupling in the juvenile frog and the underlying neural pathways to understand how gaze control processes are altered in accordance with the animal's change in body plan and locomotor strategy. Recordings of extraocular and limb motor nerves during spontaneous “fictive” swimming in isolated CNS preparations revealed that there is indeed a corresponding change in spinal efference copy control of extraocular motor output. In contrast to fictive larval swimming where alternating bursts occur in bilateral antagonistic horizontal extraocular nerves, during adult fictive limb-kicking, these motor nerves are synchronously active in accordance with the production of convergent eye movements during the linear head accelerations resulting from forward propulsion. Correspondingly, the neural pathways mediating spino-extraocular coupling have switched from contralateral to strictly ipsilateral ascending influences that ensure a coactivation of bilateral extraocular motoneurons with synchronous left–right limb extensions. Thus, adaptive developmental plasticity during metamorphosis enables spinal CPG-driven extraocular motor activity to match the changing requirements for eye movement control during self-motion.

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Adaptation to Speed in Macaque Middle Temporal and Medial Superior Temporal Areas

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The response of a sensory neuron to an unchanging stimulus typically adapts, showing decreases in response gain that are accompanied by changes in the shape of tuning curves. It remains unclear whether these changes arise purely due to spike rate adaptation within single neurons or whether they are dependent on network interactions between neurons. Further, it is unclear how the timescales of neural and perceptual adaptation are related. To examine this issue, we compared speed tuning of middle temporal (MT) and medial superior temporal neurons in macaque visual cortex after adaptation to two different reference speeds. For 75% of speed-tuned units, adaptation caused significant changes in tuning that could be explained equally well as lateral shifts, vertical gain changes, or both. These tuning changes occurred rapidly, as both neuronal firing rate and Fano factor showed no evidence of changing beyond the first 500 ms after motion onset, and the magnitude of tuning curve changes showed no difference between trials with adaptation durations shorter or longer than 1 s. Importantly, the magnitude of tuning shifts was correlated with the transient-sustained index, which measures a well characterized form of rapid response adaptation in MT, and is likely associated with changes at the level of neuronal networks. Tuning curves changed in a manner that increased neuronal sensitivity around the adapting speed, consistent with improvements in human and macaque psychophysical performance that we observed over the first several hundred ms of adaptation.

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BEHAVIORAL/COGNITIVE

Multivoxel Patterns Reveal Functionally Differentiated Networks Underlying Auditory Feedback Processing of Speech

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The everyday act of speaking involves the complex processes of speech motor control. An important component of control is monitoring, detection, and processing of errors when auditory feedback does not correspond to the intended motor gesture. Here we show, using fMRI and converging operations within a multivoxel pattern analysis framework, that this sensorimotor process is supported by functionally differentiated brain networks. During scanning, a real-time speech-tracking system was used to deliver two acoustically different types of distorted auditory feedback or unaltered feedback while human participants were vocalizing monosyllabic words, and to present the same auditory stimuli while participants were passively listening. Whole-brain analysis of neural-pattern similarity revealed three functional networks that were differentially sensitive to distorted auditory feedback during vocalization, compared with during passive listening. One network of regions appears to encode an “error signal” regardless of acoustic features of the error: this network, including right angular gyrus, right supplementary motor area, and bilateral cerebellum, yielded consistent neural patterns across acoustically different, distorted feedback types, only during articulation (not during passive listening). In contrast, a frontotemporal network appears sensitive to the speech features of auditory stimuli during passive listening; this preference for speech features was diminished when the same stimuli were presented as auditory concomitants of vocalization. A third network, showing a distinct functional pattern from the other two, appears to capture aspects of both neural response profiles. Together, our findings suggest that auditory feedback processing during speech motor control may rely on multiple, interactive, functionally differentiated neural systems.

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The Rostral Medial Prefrontal Cortex Regulates the Expression of Conditioned Eyelid Responses in Behaving Rabbits

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We studied the contribution of the rostral mPFC (rmPFC) to the acquisition and performance of classical eyeblink conditioning in rabbits using a delay paradigm. The rmPFC was determined by its afferent projections from the medial half of the mediodorsal thalamic nucleus. The rmPFC neurons were identified by their antidromic activation from the mediodorsal nucleus and/or by their firing characteristics. The rmPFC neurons increased their firing during the first conditioning sessions, but decreased it when conditioned responses (CRs) reached asymptotic values. Therefore, no significant relationships could be established between neuronal firing rates and the percentage of CRs or the electromyographic (EMG) activity of the orbicularis oculi muscle during conditioning. Electrical train stimulation of the rmPFC produced a significant inhibition of air-puff-evoked blinks and reduced the generation of CRs compared with controls. Inhibition of the rmPFC by the local injection of lidocaine produced an increase in the amplitude of evoked reflex and conditioned eyeblinks and in the percentage of CRs. The rmPFC seems to be a potent inhibitor of reflex and conditioned eyeblinks, controlling the release of newly acquired eyelid responses until advanced stages of the acquisition process—i.e., until the need for the acquired response is fully confirmed. Therefore, the rmPFC seems to act as a “flip-flop” mechanism in controlling behavior.

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Illusory Movement of Stationary Stimuli in the Visual Periphery: Evidence for a Strong Centrifugal Prior in Motion Processing

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Visual input is remarkably diverse. Certain sensory inputs are more probable than others, mirroring statistical regularities of the visual environment. The visual system exploits many of these regularities, resulting, on average, in better inferences about visual stimuli. However, by incorporating prior knowledge into perceptual decisions, visual processing can also result in perceptions that do not match sensory inputs. Such perceptual biases can often reveal unique insights into underlying mechanisms and computations. For example, a prior assumption that objects move slowly can explain a wide range of motion phenomena. The prior on slow speed is usually rationalized by its match with visual input, which typically includes stationary or slow moving objects. However, this only holds for foveal and parafoveal stimulation. The visual periphery tends to be exposed to faster motions, which are biased toward centrifugal directions. Thus, if prior assumptions derive from experience, peripheral motion processing should be biased toward centrifugal speeds. Here, in experiments with human participants, we support this hypothesis and report a novel visual illusion where stationary objects in the visual periphery are perceived as moving centrifugally, while objects moving as fast as 7°/s toward fovea are perceived as stationary. These behavioral results were quantitatively explained by a Bayesian observer that has a strong centrifugal prior. This prior is consistent with both the prevalence of centrifugal motions in the visual periphery and a centrifugal bias of direction tuning in cortical area MT, supporting the notion that visual processing mirrors its input statistics.

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Reward Prediction Error Signal Enhanced by Striatum–Amygdala Interaction Explains the Acceleration of Probabilistic Reward Learning by Emotion

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Learning does not only depend on rationality, because real-life learning cannot be isolated from emotion or social factors. Therefore, it is intriguing to determine how emotion changes learning, and to identify which neural substrates underlie this interaction. Here, we show that the task-independent presentation of an emotional face before a reward-predicting cue increases the speed of cue–reward association learning in human subjects compared with trials in which a neutral face is presented. This phenomenon was attributable to an increase in the learning rate, which regulates reward prediction errors. Parallel to these behavioral findings, functional magnetic resonance imaging demonstrated that presentation of an emotional face enhanced reward prediction error (RPE) signal in the ventral striatum. In addition, we also found a functional link between this enhanced RPE signal and increased activity in the amygdala following presentation of an emotional face. Thus, this study revealed an acceleration of cue–reward association learning by emotion, and underscored a role of striatum–amygdala interactions in the modulation of the reward prediction errors by emotion.

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The Critical Role of Sleep Spindles in Hippocampal-Dependent Memory: A Pharmacology Study

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An important function of sleep is the consolidation of memories, and features of sleep, such as rapid eye movement (REM) or sleep spindles, have been shown to correlate with improvements in discrete memory domains. Because of the methodological difficulties in modulating sleep, however, a causal link between specific sleep features and human memory consolidation is lacking. Here, we experimentally manipulated specific sleep features during a daytime nap via direct pharmacological intervention. Using zolpidem (Ambien), a short-acting GABA_A agonist hypnotic, we show increased sleep spindle density and decreased REM sleep compared with placebo and sodium oxybate (Xyrem). Naps with increased spindles produced significantly better verbal memory and significantly worse perceptual learning but did not affect motor learning. The experimental spindles were similar to control spindles in amplitude and frequency, suggesting that the experimental intervention enhanced normal sleep processes. Furthermore, using statistical methods, we demonstrate for the first time a critical role of spindles in human hippocampal memory performance. The gains in memory consolidation exceed sleep-alone or control conditions and demonstrate the potential for targeted, exceptional memory enhancement in healthy adults with pharmacologically modified sleep.

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Fear Extinction Memory Consolidation Requires Potentiation of Pontine-Wave Activity during REM Sleep

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Sleep plays an important role in memory consolidation within multiple memory systems including contextual fear extinction memory, but little is known about the mechanisms that underlie this process. Here, we show that fear extinction training in rats, which extinguished conditioned fear, increased both slow-wave sleep and rapid-eye movement (REM) sleep. Surprisingly, 24 h later, during memory testing, only 57% of the fear-extinguished animals retained fear extinction memory. We found that these animals exhibited an increase in phasic pontine-wave (P-wave) activity during post-training REM sleep, which was absent in the 43% of animals that failed to retain fear extinction memory. The results of this study provide evidence that brainstem activation, specifically potentiation of phasic P-wave activity, during post-training REM sleep is critical for consolidation of fear extinction memory. The results of this study also suggest that, contrary to the popular hypothesis of sleep and memory, increased sleep after training alone does not guarantee consolidation and/or retention of fear extinction memory. Rather, the potentiation of specific sleep-dependent physiological events may be a more accurate predictor for successful consolidation of fear extinction memory. Identification of this unique mechanism will significantly improve our present understanding of the cellular and molecular mechanisms that underlie the sleep-dependent regulation of emotional memory. Additionally, this discovery may also initiate development of a new, more targeted treatment method for clinical disorders of fear and anxiety in humans that is more efficacious than existing methods such as exposure therapy that incorporate only fear extinction.

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A Developmental Shift from Positive to Negative Connectivity in Human Amygdala–Prefrontal Circuitry

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Recent human imaging and animal studies highlight the importance of frontoamygdala circuitry in the regulation of emotional behavior and its disruption in anxiety-related disorders. Although tracing studies have suggested changes in amygdala–cortical connectivity through the adolescent period in rodents, less is known about the reciprocal connections within this circuitry across human development, when these circuits are being fine-tuned and substantial changes in emotional control are observed. The present study examined developmental changes in amygdala–prefrontal circuitry across the ages of 4–22 years using task-based functional magnetic resonance imaging. Results suggest positive amygdala–prefrontal connectivity in early childhood that switches to negative functional connectivity during the transition to adolescence. Amygdala–medial prefrontal cortex functional connectivity was significantly positive (greater than zero) among participants younger than 10 years, whereas functional connectivity was significantly negative (less than zero) among participants 10 years and older, over and above the effect of amygdala reactivity. The developmental switch in functional connectivity was paralleled by a steady decline in amygdala reactivity. Moreover, the valence switch might explain age-related improvement in task performance and a developmentally normative decline in anxiety. Initial positive connectivity followed by a valence shift to negative connectivity provides a neurobiological basis for regulatory development and may present novel insight into a more general process of developing regulatory connections.

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Brain Changes Associated with Postural Training in Patients with Cerebellar Degeneration: A Voxel-Based Morphometry Study

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Recent research indicates that physiotherapy can improve motor performance of patients with cerebellar degeneration. Given the known contributions of the cerebellum to motor learning, it remains unclear whether such observable changes in performance are mediated by the cerebellum or cerebral brain areas involved in motor control and learning. The current study addressed this question by assessing the increase in gray matter volume due to sensorimotor training in cerebellar patients using voxel-based morphometry. Nineteen human subjects with pure cerebellar degeneration and matched healthy controls were trained for 2 weeks on a balance task. Postural and clinical assessments along with structural magnetic resonance imaging were performed pretraining and post-training. The main findings were as follows. First, training enhanced balance performance in cerebellar patients. Second, in contrast to controls patients revealed significantly more post-training gray matter volume in the dorsal premotor cortex. Third, training-related increase in gray matter volume was observed within the cerebellum and was more pronounced in controls than in patients. However, statistically cerebellar changes were at the trend level and thus require additional, independent confirmation. We conclude that sensorimotor training of patients with cerebellar neurodegeneration induces gray matter changes primarily within nonaffected neocortical regions of the cerebellar-cortical loop. Residual function of the cerebellum appears to be exploited suggesting either a recovery from degeneration or intact processes of cerebellar plasticity in the remaining healthy tissue.

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Protein Phosphatase-Dependent Circadian Regulation of Intermediate-Term Associative Memory

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The endogenous circadian clock is a principal factor modulating memory across species. Determining the processes through which the circadian clock modulates memory formation is a key issue in understanding and identifying mechanisms to improve memory. We used the marine mollusk *Aplysia californica* to investigate circadian modulation of intermediate-term memory (ITM) and the mechanisms through which the circadian clock phase specifically suppresses memory using the operant learning paradigm, learning that food is inedible. We found that ITM, a temporally and mechanistically distinct form of memory, is rhythmically expressed under light-dark and constant conditions when induced by either massed or spaced training. Strong circadian regulation of ITM occurs with memory exhibited only by animals trained during the early subjective day; no apparent memory is expressed when training occurs during the late subjective day or night. Given the necessity of multiple persistent kinase cascades for ITM, we investigated whether protein phosphatase activity affected circadian modulation. Inhibition of protein phosphatases 1 and 2A blocked ITM when animals were trained during the early (subjective) day while resulting in phase-specific memory rescue when animals were trained late in the subjective day and early night. In contrast, inhibition of calcineurin did not block ITM when animals were trained during the early day and permitted ITM when animals were trained during the late subjective day, early evening, and throughout the night. These results demonstrate that levels of protein phosphatase activity are critical regulators of ITM and one mechanism through which the circadian clock regulates memory formation.

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NEUROBIOLOGY OF DISEASE

Microglia Regulate the Number of Neural Precursor Cells in the Developing Cerebral Cortex

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Neurogenesis must be properly regulated to ensure that cell production does not exceed the requirements of the growing cerebral cortex, yet our understanding of mechanisms that restrain neuron production remains incomplete. We investigated the function of microglial cells in the developing cerebral cortex of prenatal and postnatal macaques and rats and show that microglia limit the production of cortical neurons by phagocytosing neural precursor cells. We show that microglia selectively colonize the cortical proliferative zones and phagocytose neural precursor cells as neurogenesis nears completion. We found that deactivating microglia *in utero* with tetracyclines or eliminating microglia from the fetal cerebral cortex with liposomal clodronate significantly increased the number of neural precursor cells, while activating microglia *in utero* through maternal immune activation significantly decreased the number of neural precursor cells. These data demonstrate that microglia play a fundamental role in regulating the size of the precursor cell pool in the developing cerebral cortex, expanding our understanding of the mechanisms that regulate cortical development. Furthermore, our data suggest that any factor that alters the number or activation state of microglia *in utero* can profoundly affect neural development and affect behavioral outcomes.

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Shift from Extracellular Signal-Regulated Kinase to AKT/cAMP Response Element-Binding Protein Pathway Increases Survival-Motor-Neuron Expression in Spinal-Muscular-Atrophy-Like Mice and Patient Cells

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Spinal muscular atrophy (SMA), a recessive neurodegenerative disease, is characterized by the selective loss of spinal motor neurons. No available therapy exists for SMA, which represents one of the leading genetic causes of death in childhood. SMA is caused by a mutation of the survival-of-motor-neuron 1 (*SMN1*) gene, leading to a quantitative defect in the survival-motor-neuron (SMN) protein expression. All patients retain one or more copies of the *SMN2* gene, which modulates the disease severity by producing a small amount of stable SMN protein. We reported recently that NMDA receptor activation, directly in the spinal cord, significantly enhanced the transcription rate of the *SMN2* genes in a mouse model of very severe SMA (referred as type 1) by a mechanism that involved AKT/CREB pathway activation. Here, we provide the first compelling evidence for a competition between the MEK/ERK/Elk-1 and the phosphatidylinositol 3-kinase/AKT/CREB signaling pathways for *SMN2* gene regulation in the spinal cord of type 1 SMA-like mice. The inhibition of the MEK/ERK/Elk-1 pathway promotes the AKT/CREB pathway activation, leading to (1) an enhanced SMN expression in the spinal cord of SMA-like mice and in human SMA myotubes and (2) a 2.8-fold lifespan extension in SMA-like mice. Furthermore, we identified a crosstalk between ERK and AKT signaling pathways that involves the calcium-dependent modulation of CaMKII activity. Together, all these data open new perspectives to the therapeutic strategy for SMA patients.

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Behavioral and Structural Responses to Chronic Cocaine Require a Feedforward Loop Involving Δ FosB and Calcium/Calmodulin-Dependent Protein Kinase II in the Nucleus Accumbens Shell

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The transcription factor Δ FosB and the brain-enriched calcium/calmodulin-dependent protein kinase II (CaMKII α) are induced in the nucleus accumbens (NAc) by chronic exposure to cocaine or other psychostimulant drugs of abuse, in which the two proteins mediate sensitized drug responses. Although Δ FosB and CaMKII α both regulate AMPA glutamate receptor expression and function in NAc, dendritic spine formation on NAc medium spiny neurons (MSNs), and locomotor sensitization to cocaine, no direct link between these molecules has to date been explored. Here, we demonstrate that Δ FosB is phosphorylated by CaMKII α at the protein-stabilizing Ser27 and that CaMKII is required for the cocaine-mediated accumulation of Δ FosB in rat NAc. Conversely, we show that Δ FosB is both necessary and sufficient for cocaine induction of CaMKII α gene expression *in vivo*, an effect selective for D₁-type MSNs in the NAc shell subregion. Furthermore, induction of dendritic spines on NAc MSNs and increased behavioral responsiveness to cocaine after NAc overexpression of Δ FosB are both CaMKII dependent. Importantly, we demonstrate for the first time induction of Δ FosB and CaMKII in the NAc of human cocaine addicts, suggesting possible targets for future therapeutic intervention. These data establish that Δ FosB and CaMKII engage in a cell-type- and brain-region-specific positive feedforward loop as a key mechanism for regulating the reward circuitry of the brain in response to chronic cocaine.

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Adenosine Transporter ENT1 Regulates the Acquisition of Goal-Directed Behavior and Ethanol Drinking through A_{2A} Receptor in the Dorsomedial Striatum

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Adenosine signaling has been implicated in the pathophysiology of many psychiatric disorders including alcoholism. Striatal adenosine A_{2A} receptors (A_{2A}R) play an essential role in both ethanol drinking and the shift from goal-directed action to habitual behavior. However, direct evidence for a role of striatal A_{2A}R signaling in ethanol drinking and habit development has not been established. In the present study, we found that decreased A_{2A}R-mediated CREB activity in the dorsomedial striatum (DMS) enhanced initial behavioral acquisition of goal-directed behaviors and the vulnerability to progress to excessive ethanol drinking during operant conditioning in mice

lacking ethanol-sensitive adenosine transporter ENT1 (ENT1^{-/-}). Using mice expressing β -galactosidase (lacZ) under the control of seven repeated CRE sites in both genotypes (CRE-lacZ/ENT1^{+/+} mice and CRE-lacZ/ENT1^{-/-} mice) and the dominant-negative form of CREB, we found that reduced CREB activity in the DMS was causally associated with decreased A_{2A}R signaling and increased goal-directed ethanol drinking. Finally, we have demonstrated that the A_{2A}R antagonist ZM241385 dampened protein kinase A activity-mediated signaling in the DMS and promoted excessive ethanol drinking in ENT1^{+/+} mice, but not in ENT1^{-/-} mice. Our results indicate that A_{2A}R-mediated CREB signaling in the DMS is a key determinant in enhancing the development of goal-directed ethanol drinking in mice.

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Deletion of *Abca7* Increases Cerebral Amyloid- β Accumulation in the J20 Mouse Model of Alzheimer's Disease

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ATP-binding cassette transporter A7 (ABCA7) is expressed in the brain and has been detected in macrophages, microglia, and neurons. ABCA7 promotes efflux of lipids from cells to apolipoproteins and can also regulate phagocytosis and modulate processing of amyloid precursor protein (APP) to generate the Alzheimer's disease (AD) amyloid- β (A β) peptide. Genome-wide association studies have indicated that *ABCA7* single nucleotide polymorphisms confer increased risk for late-onset AD; however, the role that ABCA7 plays in the brain in the AD context is unknown. In the present study, we crossed ABCA7-deficient (A7^{-/-}) mice with J20 amyloidogenic mice to address this issue. We show that ABCA7 loss doubled insoluble A β levels and thioflavine-S-positive plaques in the brain. This was not related to changes in APP processing (assessed by analysis of full-length APP and the APP β C-terminal fragment). Apolipoprotein E regulates cerebral A β homeostasis and plaque load; however, the apolipoprotein E concentration was not altered by ABCA7 loss. Spatial reference memory was significantly impaired in both J20 and J20/A7^{-/-} mice compared with wild-type mice; however, there were no cognitive differences between J20 and J20/A7^{-/-} mice. There were also no major differences detected in hippocampal or plaque-associated microglial/macrophage markers between J20 and J20/A7^{-/-} mice, whereas the capacity for bone marrow-derived macrophages derived from A7^{-/-} mice to take up oligomeric A β was reduced by 51% compared with wild-type mice. Our results suggest that ABCA7 plays a role in the regulation of A β homeostasis in the brain and that this may be related to altered phagocyte function.

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Cortical–Amygdalar Circuit Dysfunction in a Genetic Mouse Model of Serotonin Deficiency

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Although the majority of first-line antidepressants increase brain serotonin and rare polymorphisms in tryptophan hydroxylase-2 (Tph2), the rate-limiting enzyme in the brain serotonin synthesis pathway, have been identified in cohorts of subjects with major depressive disorder, the circuit level alterations that result from serotonergic hypofunction remain poorly understood. Here we use chronic multicircuit neurophysiological recordings to characterize functional interactions across cortical and limbic circuits in mice engineered to express a human loss-of-function depression allele *Tph2*-(R441H) [Tph2 knockin (Tph2KI)]. Our results show that Tph2KI mice exhibit increased intra-network synchrony within medial prefrontal cortex (mPFC) and basal amygdala (AMY) and increased inter-network synchrony between these two brain networks. Moreover, we demonstrate that chronic treatment with fluoxetine reverses several of the circuit alterations observed within Tph2KI mice. Together, our findings establish a functional link between functional hyposerotonergia and altered mPFC–AMY network dynamics.

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Predominance of D2 Receptors in Mediating Dopamine's Effects in Brain Metabolism: Effects of Alcoholism

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Dopamine signals through D1-like and D2-like receptors, which can stimulate or inhibit, respectively, neuronal activity. Here we assessed the balance between D1 or D2 receptor signaling in the human brain and how it is affected in alcoholism. Using PET, we measured the relationship between changes in dopamine and brain glucose metabolism induced by methylphenidate in controls and alcoholics. We show that methylphenidate induced significant DA increases in striatum, amygdala, and medial orbitofrontal cortex, whereas it decreased metabolism in these brain regions. Methylphenidate-induced dopamine increases were greater in controls than in alcoholics, whereas methylphenidate-induced metabolic decreases were greater in alcoholics. For both groups, methylphenidate-induced dopamine increases were associated with decreases in regional brain metabolism, and the correlations were strongest in subthalamic nuclei, anterior cingulate, and medial orbitofrontal cortex. These correlations were more extensive and robust and the slopes steeper in alcoholics than in controls despite their attenuated dopamine responses to methylphenidate, which suggests an

impaired modulation of dopamine signals in the brain of alcoholic subjects. These findings are consistent with a predominant inhibitory effect of dopamine in the human brain that is likely mediated by the prominence of dopamine D2/D3 receptors.
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Parkinson's Disease: Increased Motor Network Activity in the Absence of Movement

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We used a network approach to assess systems-level abnormalities in motor activation in humans with Parkinson's disease (PD). This was done by measuring the expression of the normal movement-related activation pattern (NMRP), a previously validated activation network deployed by healthy subjects during motor performance. In this study, NMRP expression was prospectively quantified in ^{15}O -water PET scans from a PD patient cohort comprised of a longitudinal early-stage group ($n = 12$) scanned at baseline and at two or three follow-up visits two years apart, and a moderately advanced group scanned on and off treatment with either subthalamic nucleus deep brain stimulation ($n = 14$) or intravenous levodopa infusion ($n = 14$). For each subject and condition, we measured NMRP expression during both movement and rest. Resting expression of the abnormal PD-related metabolic covariance pattern was likewise determined in the same subjects. NMRP expression was abnormally elevated ($p < 0.001$) in PD patients scanned in the nonmovement rest state. By contrast, network activity measured during movement did not differ from normal ($p = 0.34$). In the longitudinal cohort, abnormal increases in resting NMRP expression were evident at the earliest clinical stages ($p < 0.05$), which progressed significantly over time ($p = 0.003$). Analogous network changes were present at baseline in the treatment cohort ($p = 0.001$). These abnormalities improved with subthalamic nucleus stimulation ($p < 0.005$) but not levodopa ($p = 0.25$). In both cohorts, the changes in NMRP expression that were observed did not correlate with concurrent PD-related metabolic covariance pattern measurements ($p > 0.22$). Thus, the resting state in PD is characterized by changes in the activity of normal as well as pathological brain networks.

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