

This Week in The Journal

● Cellular/Molecular

Transcriptional Regulation of Acetylcholinesterase

Shelley Camp, Antonella De Jaco, Limin Zhang, Michael Marquez, Brian De La Torre, and Palmer Taylor

(see pages 2459–2470)

Acetylcholinesterase (AChE) expression increases dramatically as myoblasts and neuroblasts differentiate respectively into myotubes and neurons, but the molecular regulators responsible for this switch have not been identified. By expressing the entire *AChE* gene (with successive deletions) in a myoblast cell line, Camp et al. have now identified a region necessary for upregulation of AChE in muscle cells. Remarkably, the regulatory region is not necessary for expression of AChE in neurons. Upregulation of AChE in muscles required the endogenous promoter as well as this regulatory region—which includes consensus binding sites for the muscle regulatory factors MyoD and MEF2—suggesting that the region constitutes an enhancer. When the regulatory region was removed by homologous recombination in transgenic mice, AChE expression was normal in neurons but almost completely absent from muscles and neuromuscular junctions. This provides the first strong evidence that AChE at neuromuscular junctions is produced in the muscle rather than motor neurons.

▲ Development/Plasticity/Repair

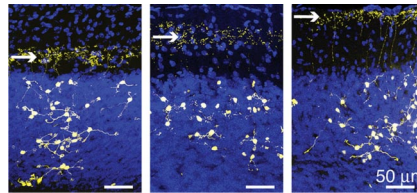
Mosaic Analysis of Cerebellar Development

J. Sebastian Espinosa and Liqun Luo

(see pages 2301–2312)

Mosaic analysis with double markers is a recently developed technique that can be used to label the progeny of single precursor cells. Espinosa and Luo used this technique to examine the proliferation and differentiation of cerebellar granule cells. Initial work revealed that all the daughters of a single granule cell precursor extend axons in a single sublayer of the molecular

layer. The authors now show that although proliferation of precursors generally slows during development, individual precursors and their progeny begin dividing rapidly (and symmetrically) just before differentiation. These neurons form a clonally related group that exits the cell cycle together. As these cells differentiate, their axons extend along the surface of the developing molecular layer, creating a cluster of axons in one sublayer. This is significant because axons in different sublayers synapse onto different interneurons and different regions of Purkinje cells, and therefore clonally related cells may also be functionally related.



Clonally related cells (yellow) extend axons in single sublayers of the molecular layer (arrows).

■ Behavioral/Systems/Cognitive

Cannabinoid Effects on Threat Perception

K. Luan Phan, Mike Angstadt, Jamie Golden, Ikechukwu Onyewuenyi, Ana Popovska, and Harriet de Wit

(see pages 2313–2319)

Cannabinoid receptors are expressed at high levels in the amygdala, where they are thought to play a role in reducing anxiety and extinguishing fear. Although many experimenters report anxiolytic effects of Δ^9 -tetrahydrocannabinol (THC), the neural basis for these subjective effects has not been demonstrated in humans. To begin to address this question, Phan et al. used functional magnetic resonance imaging to examine activity in the amygdala when subjects viewed threatening (angry or fearful) or nonthreatening (happy) faces—a probe of reactivity to social threat. As expected, control subjects had more activity in the amygdala when viewing

threatening faces than when viewing nonthreatening faces. The difference between threatening and nonthreatening faces was smaller in subjects who had taken THC. This effect depended mostly on decreased responses to threatening faces, but a small increase in responses to nonthreatening faces was also detected.

◆ Neurobiology of Disease

Conditional Model of Parkinson's Disease

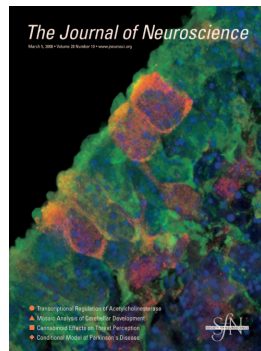
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(see pages 2471–2480)

The pathological mediators of neurodegeneration in Parkinson's disease (PD) are unknown. One hypothesis postulates that neurodegeneration results from proteolytic stress due to accumulation and aggregation of misfolded or overexpressed proteins. In support of this, mutations and duplications of the α -synuclein gene are found in some PD families, and aggregates of α -synuclein in cytoplasmic inclusions (Lewy bodies) are a hallmark of PD. This week, Nuber et al. report the development of transgenic mice that conditionally overexpress α -synuclein. The mice showed progressive loss of motor and cognitive function and some degeneration of hippocampal neurons and of dopaminergic neurons in the substantia nigra. Although cell death occurred in the substantia nigra, levels were below statistical significance, and most was not apoptotic as is seen in PD. α -Synuclein aggregated but did not form Lewy bodies. Interestingly, turning off α -synuclein overexpression slowed the progression of motor dysfunction but did not alter cellular pathology.

The Journal of Neuroscience

March 5, 2008 • Volume 28 Number 10 www.jneurosci.org



Cover legend: Newly emerging *plp*-activated neural progenitor cells at the third ventricle in the prethalamus of an E13.5 *plp-shble-lacZ* mouse embryo. β -Galactosidase antibody (in red) marks the activated progenitors; blbp (brain lipid-binding protein) antibody (in green) identifies these progenitors as radial glial cells; nuclear marker Hoechst (in blue). The picture was taken on a confocal laser microscope (Leica). For more information, see the article by Delaunay et al. in this issue (pages 2551–2562).

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A Vicious Cycle Involving Release of Heat Shock Protein 60 from Injured Cells and Activation of Toll-Like Receptor 4 Mediates Neurodegeneration in the CNS

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Infection, ischemia, trauma, and neoplasia elicit a similar inflammatory response in the CNS characterized by activation of microglia, the resident CNS monocyte. The molecular events leading from CNS injury to the activation of innate immunity is not well understood. We show here that the intracellular chaperone heat shock protein 60 (HSP60) serves as a signal of CNS injury by activating microglia through a toll-like receptor 4 (TLR4)-dependent and myeloid differentiation factor 88 (MyD88)-dependent pathway. HSP60 is released from CNS cells undergoing necrotic or apoptotic cell death and specifically binds to microglia. HSP60-induced synthesis of neurotoxic nitric oxide by microglia is dependent on TLR4. HSP60 induces extensive axonal loss and neuronal death in CNS cultures from wild-type but not TLR4 or MyD88 loss-of-function mutant mice. This is the first evidence of an endogenous molecular pathway common to many forms of neuronal injury that bidirectionally links CNS inflammation with neurodegeneration.

The Journal of Neuroscience, March 5, 2008 • 28(10):2320–2331

Expression of Nonclassical Class I Major Histocompatibility Genes Defines a Tripartite Organization of the Mouse Vomeronasal System

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The epithelium of the mouse vomeronasal organ (VNO) consists of apical and basal layers of neuronal cell bodies. Vomeronasal sensory neurons (VSNs) with cell bodies in the basal layer express the G-protein subunit $G\alpha_x$, and members of the V2R superfamily of vomeronasal receptor genes and project their axons to the posterior accessory bulb (AOB). $V2R^+$ VSNs also express particular patterns of a family of nine nonclassical class I major histocompatibility *Mhc* genes, the *H2-Mv* genes. The function of *H2-Mv* molecules remains unknown. *H2-Mv* molecules have been reported to be associated with V2R molecules and have been proposed to participate in pheromone detection. Here, we find that a substantial fraction of $V2R^+$ VSNs does not express these nine *H2-Mv* genes. The cell bodies of $H2-Mv^+$ and $H2-Mv^-$ VSNs reside in the lower and upper sublayers of the basal layer, respectively. This spatial segregation is maintained at the level of the AOB: $H2-Mv^+$ and $H2-Mv^-$ VSNs project their axons to the posterior and anterior subdomains of the posterior AOB, respectively. By generating a C-terminal green fluorescent protein fusion protein with M10.2 in gene-targeted mice, we observe subcellular localization of M10.2 not only in dendrites but also in axons of VSNs. Our results reveal a tripartite organization of the VNO and AOB, question the generality of the requirement of these nine *H2-Mv* molecules for V2R surface expression, and suggest that *H2-Mvs* can function in both dendrites and axons.

The Journal of Neuroscience, March 5, 2008 • 28(10):2332–2341

Variant Brain-Derived Neurotrophic Factor (Val66Met) Alters Adult Olfactory Bulb Neurogenesis and Spontaneous Olfactory Discrimination

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Neurogenesis, the division, migration, and differentiation of new neurons, occurs throughout life. Brain derived neurotrophic factor (BDNF) has been identified as a potential signaling molecule regulating neurogenesis in the subventricular zone (SVZ), but its functional consequences *in vivo* have not been well defined. We report marked and unexpected deficits in survival but not proliferation of newly born cells of adult knock-in mice containing a variant form of BDNF [a valine (Val) to methionine (Met) substitution at position 66 in the prodomain of BDNF (Val66Met)], a genetic mutation shown to lead to a selective impairment in activity-dependent BDNF secretion. Utilizing knock-out mouse lines, we identified BDNF and tyrosine receptor kinase B (TrkB) as the critical molecules for the observed impairments in neurogenesis, with p75 knock-out mice showing no effect on cell proliferation or survival. We then localized the activated form of TrkB to a discrete population of cells, type A migrating neuroblasts, and demonstrate a decrease in TrkB phosphorylation in the SVZ of Val66Met mutant mice. With these findings, we identify TrkB signaling, potentially through

activity dependent release of BDNF, as a critical step in the survival of migrating neuroblasts. Utilizing a behavioral task shown to be sensitive to disruptions in olfactory bulb neurogenesis, we identified specific impairments in spontaneous olfactory discrimination, but not general olfactory sensitivity or habituation to olfactory stimuli in BDNF mutant mice. Through these observations, we have identified novel links between genetic variant BDNF and adult neurogenesis *in vivo*, which may contribute to significant impairments in olfactory function.

The Journal of Neuroscience, March 5, 2008 • 28(10):2383–2393

Endothelin-1 Regulates Astrocyte Proliferation and Reactive Gliosis via a JNK/c-Jun Signaling Pathway

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Reactive gliosis is characterized by enhanced glial fibrillary acidic protein (GFAP) expression, cellular hypertrophy, and astrocyte proliferation. The cellular and molecular mechanisms underlying this process are still largely undefined. We investigated the role of endothelin-1 (ET-1) in reactive gliosis in corpus callosum after lyssolecithin (LPC)-induced focal demyelination and in cultured astrocytes. We show that ET-1 levels are upregulated in demyelinated lesions within 5 d after LPC injection, together with enhanced astrocyte proliferation, GFAP expression, and JNK phosphorylation. Infusion of the pan-ET-receptor (ET-R) antagonist Bosentan or the selective ET_B-R antagonist BQ788 into the corpus callosum prevented postlesion astrocyte proliferation and JNK phosphorylation. In cultured astrocytes, ET-1-induced activation of ET_B-Rs promotes a reactive phenotype by enhancing both GFAP expression and astrocyte proliferation. In the same cells, ET-1 activates both JNK and p38MAPK pathways, and induces c-Jun expression at the mRNA and protein levels. By using selective pharmacological inhibitors, we also provide evidence that ET-1 induces astrocyte proliferation and GFAP expression through activation of ERK- and JNK-dependent pathways, consistent with the previous observation of ET-1-induced activation of ERK (Schinelli et al., 2001). Finally, we show by gain and loss of function that increased c-Jun expression enhances the proliferative response of astrocytes to ET-1, whereas *c-jun* siRNA prevents ET-1-induced cell proliferation. Our results indicate that the effects of ET-1 on astrocyte proliferation depend on c-Jun induction and activation through ERK- and JNK-dependent pathways, and suggest that ET-R-associated pathways might represent important targets to control reactive gliosis.

The Journal of Neuroscience, March 5, 2008 • 28(10):2394–2408

Dopamine Receptor Activation Is Required for Corticostriatal Spike-Timing-Dependent Plasticity

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Single action potentials (APs) backpropagate into the higher-order dendrites of striatal spiny projection neurons during cortically driven “up” states. The timing of these backpropagating APs relative to the arriving corticostriatal excitatory inputs determines changes in dendritic calcium concentration. The question arises to whether this spike-timing relative to cortical excitatory inputs can also induce synaptic plasticity at corticostriatal synapses. Here we show that timing of single postsynaptic APs relative to the cortically evoked EPSP determines both the direction and the strength of synaptic plasticity in spiny projection neurons. Single APs occurring 30 ms before the cortically evoked EPSP induced long-term depression (LTD), whereas APs occurring 10 ms after the EPSP induced long-term potentiation (LTP). The amount of plasticity decreased as the time between the APs and EPSPs was increased, with the resulting spike-timing window being broader for LTD than for LTP. In addition, we show that dopamine receptor activation is required for this spike-timing-dependent plasticity (STDP). Blocking dopamine D₁/D₅ receptors prevented both LTD and LTP induction. In contrast, blocking dopamine D₂ receptors delayed, but did not prevent, LTD and sped induction of LTP. We conclude (1) that, in combination with cortical inputs, single APs evoked in spiny projection neurons can induce both LTP and LTD of the corticostriatal pathway; (2) that the strength and direction of these synaptic changes depend deterministically on the AP timing relative to the arriving cortical inputs; (3) that, whereas dopamine D₂ receptor activation modulates the initial phase of striatal STDP, dopamine D₁/D₅ receptor activation is critically required for striatal STDP. Thus, the timing of APs relative to cortical inputs alone is not enough to induce corticostriatal plasticity, implying that ongoing activity does not affect synaptic strength unless dopamine receptors are activated.

The Journal of Neuroscience, March 5, 2008 • 28(10):2435–2446

Generation of Eupnea and Sighs by a Spatiochemically Organized Inspiratory Network

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The discovery of the rhythmogenic pre-Bötzing complex (preBötC) inspiratory network, which remains active in a transverse brainstem slice, greatly increased the understanding of neural respiratory control. However, basic questions remain unanswered such as (1) What are the necessary and sufficient slice boundaries for a functional preBötC? (2) Is the minimal preBötC capable of reconfiguring between inspiratory-related patterns (e.g., fictive eupnea and sighs)? (3) How is preBötC activity affected by surrounding structures? Using newborn rat slices with systematically varied dimensions in physiological [K⁺] (3 mM), we found that a 175 μm thickness is sufficient for generating inspiratory-related rhythms. In 700-μm-thick slices with unilaterally exposed preBötC, a kernel <100 μm thick, centered 0.5 mm caudal to the facial nucleus, is necessary for rhythm generation. Slices containing this kernel plus caudal structures produced eupneic bursts of regular amplitude, whereas this kernel plus rostral tissue generated sighs, intermingled with eupneic bursts of variable amplitude (“eupnea-sigh pattern”). After spontaneous arrest of rhythm, substance-P or neurokinin-1 (NK1) receptor agonist induced the eupnea-sigh burst pattern in ≥250-μm-thick slices, whereas thyrotropin-releasing hormone or phosphodiesterase-4

blockers evoked the eupnea burst pattern. Endogenous rhythm was depressed by NK1 receptor antagonism. Multineuronal Ca^{2+} imaging revealed that preBötC neurons reconfigure between eupnea and eupnea–sigh burst patterns. We hypothesize a (gradient-like) spatiochemical organization of regions adjacent to the preBötC, such that a small preBötC inspiratory-related oscillator generates eupnea under the dominant influence of caudal structures or thyrotropin-releasing hormone-like transmitters but eupnea–sigh activity when the influence of rostral structures or substance-P-like transmitters predominates.

The Journal of Neuroscience, March 5, 2008 • 28(10):2447–2458

Acetylcholinesterase Expression in Muscle Is Specifically Controlled by a Promoter-Selective Enhancesome in the First Intron

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Mammalian acetylcholinesterase (*AChE*) gene expression is exquisitely regulated in target tissues and cells during differentiation. An intron located between the first and second exons governs a ~100-fold increase in AChE expression during myoblast to myotube differentiation in C2C12 cells. Regulation is confined to 255 bp of evolutionarily conserved sequence containing functional transcription factor consensus motifs that indirectly interact with the endogenous promoter. To examine control *in vivo*, this region was deleted by homologous recombination. The knock-out mouse is virtually devoid of AChE activity and its encoding mRNA in skeletal muscle, yet activities in brain and spinal cord innervating skeletal muscle are unaltered. The transcription factors MyoD and myocyte enhancer factor-2 appear to be responsible for muscle regulation. Selective control of AChE expression by this region is also found in hematopoietic lineages. Expression patterns in muscle and CNS neurons establish that virtually all AChE activity at the mammalian neuromuscular junction arises from skeletal muscle rather than from biosynthesis in the motoneuron cell body and axoplasmic transport.

The Journal of Neuroscience, March 5, 2008 • 28(10):2459–2470

Transient Receptor Potential A1 Is a Sensory Receptor for Multiple Products of Oxidative Stress

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Transient receptor potential A1 (TRPA1) is expressed in a subset of nociceptive sensory neurons where it acts as a sensor for environmental irritants, including acrolein, and some pungent plant ingredients such as allyl isothiocyanate and cinnamaldehyde. These exogenous compounds activate TRPA1 by covalent modification of cysteine residues. We have used electrophysiological methods and measurements of intracellular calcium concentration ($[\text{Ca}^{2+}]_i$) to show that TRPA1 is activated by several classes of endogenous thiol-reactive molecules. TRPA1 was activated by hydrogen peroxide (H_2O_2 ; EC_{50} , 230 μM), by endogenously occurring alkenyl aldehydes (EC_{50} : 4-hydroxynonenal 19.9 μM , 4-oxo-nonenal 1.9 μM , 4-hydroxyhexenal 38.9 μM) and by the cyclopentenone prostaglandin, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J_2 (15d-PG J_2 , EC_{50} : 5.6 μM). The effect of H_2O_2 was reversed by treatment with dithiothreitol indicating that H_2O_2 acts by promoting the formation of disulfide bonds whereas the actions of the alkenyl aldehydes and 15d-PG J_2 were not reversed, suggesting that these agents form Michael adducts. H_2O_2 and the naturally occurring alkenyl aldehydes and 15d-PG J_2 acted on a subset of isolated rat and mouse sensory neurons [$\sim 25\%$ of rat dorsal root ganglion (DRG) and $\sim 50\%$ of nodose ganglion neurons] to evoke a depolarizing inward current and an increase in $[\text{Ca}^{2+}]_i$ in TRPA1 expressing neurons. The abilities of H_2O_2 , alkenyl aldehydes and 15d-PG J_2 to raise $[\text{Ca}^{2+}]_i$ in mouse DRG neurons were greatly reduced in neurons from *trpa1*^{-/-} mice. Furthermore, intraplantar injection of either H_2O_2 or 15d-PG J_2 evoked a nocifensive/pain response in wild-type mice, but not in *trpa1*^{-/-} mice. These data demonstrate that multiple agents produced during episodes of oxidative stress can activate TRPA1 expressed in sensory neurons.

The Journal of Neuroscience, March 5, 2008 • 28(10):2485–2494

Action Potential-Independent and Nicotinic Receptor-Mediated Concerted Release of Multiple Quanta at Hippocampal CA3–Mossy Fiber Synapses

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Presynaptic action potential-independent transmitter release is a potential means of information transfer across synapses. We show that in the hippocampal mossy fiber boutons, activation of the $\alpha 7$ -subtype of nicotinic acetylcholine receptors ($\alpha 7$ -nAChRs) results in a large increase in the amplitude of spontaneous events, resulting from concerted release of multiple quanta from the mossy fiber boutons. This amplitude increase is abolished at low temperatures. Activation of $\alpha 7$ -nAChRs causes a rise in intraterminal calcium at mossy fiber boutons, involving ryanodine receptors. Regulation of concerted release requires the subsequent activation of presynaptic calcium/calmodulin-dependent protein kinase II (CaMKII). Activation of CaMKII is required to drive presynaptic action potential-independent transmission at the mossy fiber–CA3 pyramidal cell synapse. The effects of $\alpha 7$ -nAChR activation are mediated by biologically relevant doses of nicotine. Our results demonstrate a novel form of synaptic plasticity mediated by presynaptic $\alpha 7$ -nAChRs and store calcium that is temporally different and might respond to a different history of synaptic activity than that mediated by incoming action potentials.

The Journal of Neuroscience, March 5, 2008 • 28(10):2563–2575

PKC-Induced Intracellular Trafficking of Ca_v2 Precedes Its Rapid Recruitment to the Plasma Membrane

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Activation of protein kinase C (PKC) potentiates secretion in *Aplysia* peptidergic neurons, in part by inducing new sites for peptide release at growth cone terminals. The mechanisms by which ion channels are trafficked to such sites are, however, not well understood. We now show that PKC activation rapidly recruits new Ca_v2 subunits to the plasma membrane, and that recruitment is blocked by latrunculin B, an inhibitor of actin polymerization. In contrast, inhibition of microtubule polymerization selectively prevents the appearance of Ca_v2 subunits only at the distal edge of the growth cone. In resting neurons, Ca_v2-containing organelles reside in the central region of growth cones, but are absent from distal lamellipodia. After activation of PKC, these organelles are transported on microtubules to the lamellipodium. The ability to traffic to the most distal sites of channel insertion inside the lamellipodium does, therefore, not require intact actin but requires intact microtubules. Only after activation of PKC do Ca_v2 channels associate with actin and undergo insertion into the plasma membrane.

The Journal of Neuroscience, March 5, 2008 • 28(10):2601–2612

Interplay between Cyclin-Dependent Kinase 5 and Glycogen Synthase Kinase 3 β Mediated by Neuregulin Signaling Leads to Differential Effects on Tau Phosphorylation and Amyloid Precursor Protein Processing

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Cyclin-dependent kinase 5 (cdk5) and glycogen synthase kinase 3 β (GSK3 β) have been implicated in pathogenic processes associated with Alzheimer's disease because both kinases regulate tau hyperphosphorylation and enhance amyloid precursor protein (APP) processing leading to an increase in amyloid β (A β) production. Here we show that young p25 overexpressing mice have enhanced cdk5 activity but reduced GSK3 β activity attributable to phosphorylation at the inhibitory GSK3 β -serine 9 (GSK3 β -S9) site. Phosphorylation at this site was mediated by enhanced activity of the neuregulin receptor complex, ErbB, and activation of the downstream phosphatidylinositol 3 kinase/Akt pathway. Young p25 mice had elevated A β peptide levels, but phospho-tau levels were decreased overall. Thus, cdk5 appears to play a dominant role in the regulation of amyloidogenic APP processing, whereas GSK3 β plays a dominant role in overall tau phosphorylation. In older mice, GSK3 β inhibitory phosphorylation at S9 was reduced relative to young mice. A β peptide levels were still elevated but phospho-tau levels were either unchanged or showed a trend to increase, suggesting that GSK3 β activity increases with aging. Inhibition of cdk5 by a specific inhibitor reduced cdk5 activity in p25 mice, leading to reduced A β production in both young and old mice. However, in young mice, cdk5 inhibition reversed GSK3 β inhibition, leading to an increase in overall tau phosphorylation. When cdk5 inhibitor was administered to very old, nontransgenic mice, inhibition of cdk5 reduced A β levels, and phospho-tau levels showed a trend to increase. Thus, cdk5 inhibitors may not be effective in targeting tau phosphorylation in the elderly.

The Journal of Neuroscience, March 5, 2008 • 28(10):2624–2632

D₁-Like Dopamine Receptor Activation Modulates GABAergic Inhibition But Not Electrical Coupling between Neocortical Fast-Spiking Interneurons

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Dopamine, acting through D₁ receptors, is thought to play an important role in cognitive functions of the frontal cortex such as working memory. D₁ receptors are widely expressed in fast-spiking (FS) interneurons, a prominent class of inhibitory cells that exert a powerful control of neuronal firing through proximal synapses on their postsynaptic targets. FS cells are extensively mutually interconnected by both GABA_A receptor-mediated synapses and gap junction-mediated electrical synapses, and networks of FS cells play a crucial role in the generation of rhythmic synchronous activity. Although recent studies have documented the effects of dopamine modulation of neocortical synaptic connections among excitatory cells and between excitatory and various inhibitory cells, the effects of dopamine receptor activation on GABAergic and electrical interactions among FS cells is not known. To resolve this, we recorded from pairs of FS cells in the infragranular layers of mouse neocortical slices and tested the effects of D₁-like (D₁/D₅) receptor activation on these connections. We found that D₁-like receptor activation modulated GABAergic but not electrical connections between them. A D₁-like receptor agonist preserved the strength of electrical coupling but reduced the amplitude of IPSPs and IPSCs between FS cells. Our results suggest that D₁-like receptor activation has synapse-specific effects within networks of FS cells, with potential implications for the generation of rhythmic activity in the neocortex.

The Journal of Neuroscience, March 5, 2008 • 28(10):2633–2641

Timing Neurogenesis and Differentiation: Insights from Quantitative Clonal Analyses of Cerebellar Granule Cells

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The cerebellum is an excellent model system to study how developmental programs give rise to exquisite neuronal circuits in the adult brain. Here, we describe our findings regarding granule cell neurogenesis and differentiation using the MADM method (mosaic analysis with double markers) in mice. By following the development of individual granule cell clones, we show that (1) granule cell precursors (GCPs) undergo predominantly symmetric division during postnatal development; (2) clonally related granule cells (GCs) exit the cell cycle within a narrow time window and stack their axons in the molecular layer in chronological order from deep to superficial sublayers; and (3) whereas the average GCP proliferation in the external granular layer is progressively slower as development proceeds, there is a rapid expansion of GCPs shortly before clonally related GCs exit the cell cycle. These properties produce GC clones that are distinct, each having a restricted axonal projection, but that are on average similar in cell number. We discuss possible developmental mechanisms and functional implications of these findings.

The Journal of Neuroscience, March 5, 2008 • 28(10):2301–2312

Genetic Control of Circuit Function: *Vsx1* and *Irx5* Transcription Factors Regulate Contrast Adaptation in the Mouse Retina

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Transcriptional programs guide the specification of neural cell types in the developing nervous system. However, it is unclear whether such programs also control specific aspects of neural circuit function at maturity. In the mammalian retina, *Vsx1* and *Irx5* transcription factors are present in a subset of bipolar interneurons that convey signals from photoreceptors to ganglion cells. The biased expression of *Vsx1* and *Irx5* in hyperpolarizing OFF compared with depolarizing ON bipolar cells suggests that these transcription factors may selectively regulate signal processing in OFF circuits. To test this hypothesis, we generated mice lacking both *Vsx1* and *Irx5*. Bipolar cells in these mice were morphologically normal, but the expression of cell-specific markers in some OFF but not ON bipolar cells was reduced or absent. To assess visual function in *Vsx1*^{-/-}*Irx5*^{-/-} retinas, we recorded light responses from ensembles of retinal ganglion cells (RGCs). We first identified functional RGC types in control mice and describe their response properties and adaptation to temporal contrast using a simple linear–nonlinear model. We found that space–time receptive fields of RGCs are unchanged in *Vsx1*^{-/-}*Irx5*^{-/-} mice compared with control retinas. In contrast, response threshold, gain, and range were lowered in a cell-type-specific manner in OFF but not ON RGCs in *Vsx1*^{-/-}*Irx5*^{-/-} retinas. Finally, we discovered that the ability to adapt to temporal contrast is greatly reduced in OFF RGCs in the double mutant, suggesting that *Vsx1* and *Irx5* control specific aspects of visual function in circuits of the mammalian retina.

The Journal of Neuroscience, March 5, 2008 • 28(10):2342–2352

Neurite Outgrowth and *In Vivo* Sensory Innervation Mediated by a Ca_v2.2–Laminin β 2 Stop Signal

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Axons and dendrites of developing neurons establish distributed innervation patterns enabling precise discrimination in sensory systems. We describe the role of the extracellular matrix molecule, laminin β 2, interacting with the Ca_v2.2 calcium channel in establishing appropriate sensory innervation. *In vivo*, Ca_v2.2 is expressed on the growth cones of *Xenopus laevis* sensory neurites and laminin β 2 is expressed in the skin. Culturing neurons on a laminin β 2 substrate inhibits neurite outgrowth in a specific and calcium-dependent manner. Blocking signaling between laminin β 2 and Ca_v2.2 leads to increased numbers of sensory terminals *in vivo*. These findings suggest that interactions between extracellular matrix molecules and calcium channels regulate connectivity in the developing nervous system.

The Journal of Neuroscience, March 5, 2008 • 28(10):2366–2374

Early Neuronal and Glial Fate Restriction of Embryonic Neural Stem Cells

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The question of how neurons and glial cells are generated during the development of the CNS has over time led to two alternative models: either neuroepithelial cells are capable of giving rise to neurons first and to glial cells at a later stage (switching model), or they are intrinsically committed to generate one or the other (segregating model). Using the developing diencephalon as a model and by selecting a subpopulation of ventricular cells, we analyzed both *in vitro*, using clonal analysis, and *in vivo*, using inducible Cre/loxP fate mapping, the fate of neuroepithelial and radial glial cells generated at different time points during embryonic development. We found that, during neurogenic periods [embryonic day 9.5 (E9.5) to 12.5], proteolipid protein (*plp*)-expressing cells were lineage-restricted neuronal precursors, but later in embryogenesis, during gliogenic periods (E13.5 to early postnatal), *plp*-expressing cells were lineage-restricted glial precursors. In addition, we show that glial cells forming at E13.5 arise from a new pool of neuroepithelial progenitors distinct from neuronal progenitors cells, which lends support to the segregating model.

The Journal of Neuroscience, March 5, 2008 • 28(10):2551–2562

BEHAVIORAL/SYSTEMS/COGNITIVE

Cannabinoid Modulation of Amygdala Reactivity to Social Signals of Threat in Humans

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The cannabinoid (CB) system is a key neurochemical mediator of anxiety and fear learning in both animals and humans. The anxiolytic effects of Δ^9 -tetrahydrocannabinol (THC), the primary psychoactive ingredient in cannabis, are believed to be mediated through direct and selective agonism of CB₁ receptors localized within the basolateral amygdala, a critical brain region for threat perception. However, little is known about the effects of THC on amygdala reactivity in humans. We used functional magnetic resonance imaging and a well validated task to probe amygdala responses to threat signals in 16 healthy, recreational cannabis users after a double-blind crossover administration of THC or placebo. We found that THC significantly reduced amygdala reactivity to social signals of threat but did not affect activity in primary visual and motor cortex. The current findings fit well with the notion that THC and other cannabinoids may have an anxiolytic role in central mechanisms of fear behaviors and provide a rationale for exploring novel therapeutic strategies that target the cannabinoid system for disorders of anxiety and social fear.

The Journal of Neuroscience, March 5, 2008 • 28(10):2313–2319

Functional Imaging, Spatial Reconstruction, and Biophysical Analysis of a Respiratory Motor Circuit Isolated *In Vitro*

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We combined real-time calcium-based neural activity imaging with whole-cell patch-clamp recording techniques to map the spatial organization and analyze electrophysiological properties of respiratory neurons forming the circuit transmitting rhythmic drive from the pre-Bötzinger complex (pre-BötC) through premotoneurons to hypoglossal (XII) motoneurons. Inspiratory pre-BötC neurons, XII premotoneurons (preMNs), and XII motoneurons (MNs) were retrogradely labeled with Ca²⁺-sensitive dye in neonatal rat *in vitro* brainstem slices. PreMN cell bodies were arrayed dorsomedially to pre-BötC neurons with little spatial overlap; axonal projections to MNs were ipsilateral. Inspiratory MNs were distributed in dorsal and ventral subnuclei of XII. Voltage-clamp recordings revealed that two currents, persistent sodium current (NaP) and K⁺-dominated leak current (Leak), primarily contribute to preMN/MN subthreshold current–voltage relationships. NaP or Leak conductance densities in preMNs and MNs were not significantly different. We quantified preMN and MN action potential time course and spike frequency–current (*f–I*) relationships and found no significant differences in repetitive spiking dynamics, steady-state *f–I* gains, and afterpolarizing potentials. Rhythmic synaptic drive current densities were similar in preMNs and MNs. Our results indicate that, despite topographic and morphological differences, preMNs and MNs have some common intrinsic membrane, synaptic integration, and spiking properties that we postulate ensure fidelity of inspiratory drive transmission and conversion of synaptic drive into (pre)motor output. There also appears to be a common architectonic organization for some respiratory drive transmission circuits whereby many preMNs are spatially segregated from pre-BötC rhythm-generating neurons, which we hypothesize may facilitate downstream integration of convergent inputs for premotor pattern formation.

The Journal of Neuroscience, March 5, 2008 • 28(10):2353–2365

Transient D₁ Dopamine Receptor Expression on Prefrontal Cortex Projection Neurons: Relationship to Enhanced Motivational Salience of Drug Cues in Adolescence

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Adolescence is a transitional period during development that is associated with a greater likelihood of addiction to drugs than any other age. In the prefrontal cortex (PFC), D₁ dopamine receptors mediate motivational salience attribution, which plays a role in addiction. Here, we investigated the relationship of age-related D₁ dopamine receptor expression in the PFC with the maturation of cocaine place conditioning. Confocal microscopy revealed that retrogradely traced cortical output neurons to the nucleus accumbens express higher levels of D₁ receptors during adolescence compared with younger and older ages. D₁ expression does not change on GABAergic interneurons across age. Adolescent differences in D₁ expression occur independently of cortical-accumbens connectivity, which proliferates through adulthood. Behaviorally, adolescent rats are more sensitive to cocaine place conditioning than younger and older rats. However, microinjections of the D₁ antagonist SCH23390 into the PFC blocked adolescent place preferences, whereas microinjections of D₁ agonists dose-dependently increased preferences for cocaine-associated environments previously not preferred by juveniles. These results suggest that the heightened expression of D₁ receptors on cortical-accumbens projections may help explain increased sensitivity to environmental events and addictive behaviors during adolescence, whereas the paucity of D₁-expressing projections may reduce risk in juveniles.

The Journal of Neuroscience, March 5, 2008 • 28(10):2375–2382

Individual Premotor Drive Pulses, Not Time-Varying Synergies, Are the Units of Adjustment for Limb Trajectories Constructed in Spinal Cord

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Complex actions may arise by combining simple motor primitives. Our studies support individual premotor drive pulses or bursts as execution primitives in spinal cord. Alternatively, the fundamental execution primitives at the segmental level could be time-varying synergies. To distinguish these hypotheses, we examined sensory feedback effects during targeted wiping organized in spinal cord. This behavior comprises three bursts. We tested (1) whether feedback altered the structure of individual premotor drive bursts or primitives, and (2) whether feedback differentially modulated different drive bursts or pulses in the three burst sequence. At least two of the three bursts would need to always be comodulated to support a time-varying synergy. We used selective muscle vibration to control spindle feedback from a single muscle (biceps/iliofibularis). The structures of premotor drive bursts were conserved. However, biceps vibration (1) scaled the amplitudes of two bursts coactivated during the initial phase of wiping independently of one another without altering their phase, and (2) independently phase regulated the third burst but preserved its amplitude. Thus, all three bursts were regulated separately. Durations were unaffected. The independent effects depended on (1) time of vibration during wiping, (2) frequency of vibration, and (3) limb configuration. Because each of the three bursts was independently modulated, these data strongly support execution using individual premotor bursts rather than time-varying synergies at the spinal level of motor organization. Our data show that both sensory feedback and central systems of the spinal cord act in concert to adjust the individual premotor bursts in support of the straight and unimodal wiping trajectory.

The Journal of Neuroscience, March 5, 2008 • 28(10):2409–2425

Specificity of Speech Motor Learning

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The idea that the brain controls movement using a neural representation of limb dynamics has been a dominant hypothesis in motor control research for well over a decade. Speech movements offer an unusual opportunity to test this proposal by means of an examination of transfer of learning between utterances that are to varying degrees matched on kinematics. If speech learning results in a generalizable dynamics representation, then, at the least, learning should transfer when similar movements are embedded in phonetically distinct utterances. We tested this idea using three different pairs of training and transfer utterances that substantially overlap kinematically. We find that, with these stimuli, speech learning is highly contextually sensitive and fails to transfer even to utterances that involve very similar movements. Speech learning appears to be extremely local, and the specificity of learning is incompatible with the idea that speech control involves a generalized dynamics representation.

The Journal of Neuroscience, March 5, 2008 • 28(10):2426–2434

Defects in Breathing and Thermoregulation in Mice with Near-Complete Absence of Central Serotonin Neurons

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Serotonergic neurons project widely throughout the CNS and modulate many different brain functions. Particularly important, but controversial, are the contributions of serotonin (5-HT) neurons to respiratory and thermoregulatory control. To better define the roles of 5-HT neurons in breathing and thermoregulation, we took advantage of a unique conditional knock-out mouse in which *Lmx1b* is genetically deleted in *Pet1*-expressing cells (*Lmx1b*^{fl/p}), resulting in near-complete absence of central 5-HT neurons. Here, we show that the hypercapnic ventilatory response in adult *Lmx1b*^{fl/p} mice was decreased by 50% compared with wild-type mice, whereas baseline ventilation and the hypoxic ventilatory response were normal. In addition, *Lmx1b*^{fl/p} mice rapidly became hypothermic when exposed to an ambient temperature of 4°C, decreasing core temperature to 30°C within 120 min. This failure of thermoregulation was caused by impaired shivering and nonshivering thermogenesis, whereas thermosensory perception and heat conservation were normal. Finally, intracerebroventricular infusion of 5-HT stimulated baseline ventilation, and rescued the blunted hypercapnic ventilatory response. These data identify a previously unrecognized role of 5-HT neurons in the CO₂ chemoreflex, whereby they enhance the response of the rest of the respiratory network to CO₂. We conclude that the proper function of the 5-HT system is particularly important under conditions of environmental stress and contributes significantly to the hypercapnic ventilatory response and thermoregulatory cold defense.

The Journal of Neuroscience, March 5, 2008 • 28(10):2495–2505

Bötzinger Expiratory-Augmenting Neurons and the Parafacial Respiratory Group

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In neonatal rat brains *in vitro*, the rostral ventral respiratory column (rVRC) contains neurons that burst just before the phrenic nerve discharge (PND) and rebound after inspiration (pre-I neurons). These neurons, called parafacial respiratory group (pFRG), have been interpreted as a master inspiratory oscillator, an expiratory rhythm generator or simply as neonatal precursors of retrotrapezoid (RTN) chemoreceptor neurons. pFRG neurons have not been identified in adults, and their phenotype is unknown. Here, we confirm that the rVRC normally lacks pre-I neurons in adult anesthetized rats. However, we show that, during hypercapnic hypoxia, a population of rVRC expiratory-augmenting (E-AUG) neurons consistently develops a pre-I discharge. These cells reside in the Bötzinger region of the rVRC, they express glycine-transporter-2, and their axons arborize throughout the VRC. Hypoxia triggers an identical pre-I pattern in retroambigular expiratory bulbospinal neurons, but this pattern is not elicited in Bötzinger expiratory-decrementing neurons, Bötzinger inspiratory neurons, RTN neurons, and blood pressure-regulating neurons. In conclusion, under hypoxia *in vivo*, abdominal expiratory premotor neurons of adult rats develop a pre-I pattern reminiscent of that observed in neonate brainstems *in vitro*. In the rVRC of adult rats, pre-I cells include selected rhythmic neurons (glycinergic Bötzinger neurons) but not RTN chemoreceptors. We suggest that the pFRG may not be an independent rhythm generator but a heterogeneous collection of E-AUG neurons (glycinergic Bötzinger neurons, possibly facial motor and premotor neurons), the discharge of which becomes preinspiratory under specific experimental conditions resulting from, in part, a prolonged and intensified activity of postinspiratory neurons.

The Journal of Neuroscience, March 5, 2008 • 28(10):2506–2515

Withdrawal from Cocaine Self-Administration Normalizes Deficits in Proliferation and Enhances Maturity of Adult-Generated Hippocampal Neurons

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Relapse, a major problem in the treatment of cocaine addiction, is proposed to result in part from neuroadaptations in the hippocampus. We examined how a mediator of hippocampal neuroplasticity, adult neurogenesis in the subgranular zone (SGZ), was regulated by cocaine self-administration (CSA), and whether these changes were reversed by 4 weeks of withdrawal (CSA-WD) versus continued cocaine self-administration (CSA-CONT). Rats self-administered intravenous cocaine or saline for 3 weeks and were killed 2 h (CSA) or 4 weeks (CSA-WD, CSA-CONT) after injection with the S-phase marker bromodeoxyuridine (BrdU). Cells in several stages of adult neurogenesis were quantified: proliferating cells labeled by BrdU (2 h) or Ki-67; immature neurons labeled by doublecortin; and adult-generated neurons labeled with BrdU (4 weeks) and the mature neuronal marker NeuN. CSA decreased proliferation in both the SGZ and the subventricular zone (SVZ), a source of adult-generated olfactory neurons, changes reversed by CSA-WD. Unexpectedly, CSA-WD and CSA-CONT resulted in more immature doublecortin-immunopositive (+) neurons in the posterior SGZ and a normal number of adult-generated BrdU+ neurons in the SGZ, suggesting an enduring impact of CSA regardless of whether cocaine intake was stopped or continued. However, only CSA-WD rats had more adult-generated neurons with punctate BrdU staining, an indicator of enhanced maturity. These data suggest a mechanism for the cognitive and olfactory deficits seen in cocaine addicts, and further suggest that adult-generated neurons should be considered for their potential role in cocaine addiction and hippocampal-mediated relapse after cocaine withdrawal.

The Journal of Neuroscience, March 5, 2008 • 28(10):2516–2526

A Hierarchy of Temporal Receptive Windows in Human Cortex

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Real-world events unfold at different time scales and, therefore, cognitive and neuronal processes must likewise occur at different time scales. We present a novel procedure that identifies brain regions responsive to sensory information accumulated over different time scales. We measured functional magnetic resonance imaging activity while observers viewed silent films presented forward, backward, or piecewise-scrambled in time. Early visual areas (e.g., primary visual cortex and the motion-sensitive area MT+) exhibited high response reliability regardless of disruptions in temporal structure. In contrast, the reliability of responses in several higher brain areas, including the superior temporal sulcus (STS), precuneus, posterior lateral sulcus (LS), temporal parietal junction (TPJ), and frontal eye field (FEF), was affected by information accumulated over longer time scales. These regions showed highly reproducible responses for repeated forward, but not for backward or piecewise-scrambled presentations. Moreover, these regions exhibited marked differences in temporal characteristics, with LS, TPJ, and FEF responses depending on information accumulated over longer durations (~36 s) than STS and precuneus (~12 s). We conclude that, similar to the known cortical hierarchy of spatial receptive fields, there is a hierarchy of progressively longer temporal receptive windows in the human brain.

The Journal of Neuroscience, March 5, 2008 • 28(10):2539–2550

Effector Immediate-Early Gene Arc in the Amygdala Plays a Critical Role in Alcoholism

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The immediate early gene, activity-regulated cytoskeleton-associated protein (Arc), has been implicated in synaptic plasticity. However, the role of Arc in alcoholism is unknown. Here, we report that the anxiolytic effects of acute ethanol were associated with increased brain-derived neurotrophic factor (BDNF) and tyrosine kinase B (trkB) expression, increased phosphorylation of extracellular signal-regulated kinases 1/2 (Erk1/2), Elk-1, and cAMP responsive element-binding protein (CREB), increased Arc expression, and increased dendritic spine density (DSD) in both the central amygdala (CeA) and medial amygdala (MeA) but not in the basolateral amygdala (BLA) of rats. Conversely, the anxiogenic effects of withdrawal after long-term ethanol exposure were associated with decreased BDNF and trkB expression, decreased phosphorylation of Erk1/2, Elk-1, and CREB, decreased Arc expression, and decreased DSD in both the CeA and MeA but not in the BLA of rats. We also showed that BDNF infusion into the CeA normalized phosphorylation of Erk1/2, Elk-1, and CREB, and normalized Arc expression, thereby protecting against the onset of ethanol withdrawal-related anxiety. We further demonstrated that arresting Arc expression in the CeA decreased DSD, thereby increasing anxiety-like and alcohol-drinking behaviors in control rats. These results revealed that BDNF–Arc signaling and the associated DSD in the CeA, and possibly in the MeA, may be involved in the molecular processes of alcohol dependence and comorbidity of anxiety and alcohol-drinking behaviors.

The Journal of Neuroscience, March 5, 2008 • 28(10):2589–2600

Bottom-Up Activation of the Vocal Motor Forebrain by the Respiratory Brainstem

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Brainstem motor structures send output commands to the periphery and are dynamically modulated by telencephalic inputs. Little is known, however, about ascending brainstem control of forebrain motor structures. Here, we provide the first evidence for bottom-up activation of forebrain motor centers by the respiratory brainstem. We show that, in the avian vocal control system, activation of the brainstem inspiratory nucleus paraambigualus (PAm), a likely homolog of the mammalian rostral ventral respiratory group, can drive neural activity bilaterally in the forebrain vocal control nuclei HVC (used as a proper name) and the robust nucleus of the arcopallium (RA). Furthermore, this activation is abolished by lesions of nucleus uvaeformis (Uva), a thalamic nucleus necessary for song production. We identify a type of bursting neuron within PAm whose activity is correlated, in an Uva dependent manner, to bursting activity in RA, rather than to the respiratory rhythm, and is robustly active during the production of stimulus evoked vocalizations. Because this ascending input results in cross-hemisphere activation, our results suggest a crucial role for the respiratory brainstem in coordinating forebrain motor centers during vocal production.

The Journal of Neuroscience, March 5, 2008 • 28(10):2613–2623

Cholinergic Deafferentation of Prefrontal Cortex Increases Sensitivity to Cross-Modal Distractors during a Sustained Attention Task

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The effects of restricted cholinergic deafferentation of prefrontal cortex in rats on sustained attention were assessed. Attentional demands were increased by presentation of distractor stimuli in a different modality (auditory) or the same modality (visual) as target stimuli. Additionally, the effects of the regularity of the distractor on rats' ability to disregard this stimulus were assessed by testing different frequencies of stimuli for each modality. Cholinergically lesioned rats were more sensitive to the effects

of auditory distractors than nonlesioned rats, whereas visual distractors of any frequency potently impaired the performance of all subjects. The effects of the auditory stimuli on attentional performance varied depending on the frequency of the tone. A tone with a predictable pattern enhanced signal detection in all rats. An irregular tone selectively impaired performance of rats with cholinergic lesions. Additional tests suggest that rats use the regular tone to time when to attend. Lesioned rats were impaired when the regular tone was presented with a more variable intertrial interval in a subsequent testing session, suggesting impairments in top-down control. In addition to changes in top-down control of attention, differential effects on performance based on the regularity of the tone suggest that stimulus properties encoded by bottom-up processes are also altered after lesioning. The current data suggest that cholinergic deafferentation of prefrontal cortex alters top-down and bottom-up processing of stimuli.

The Journal of Neuroscience, March 5, 2008 • 28(10):2642–2650

Extinction of a Classically Conditioned Response: Red Nucleus and Interpositus

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It is well established that the cerebellum and its associated circuitry are essential for classical conditioning of the eyeblink response and other discrete motor responses (e.g., limb flexion, head turn, etc.) learned with an aversive unconditioned stimulus. However, brain mechanisms underlying extinction of these responses are still relatively unclear. Behavioral studies have demonstrated extinction to be an active learning process distinct from acquisition. Accordingly, this current understanding of extinction has guided neural studies that have tried to identify possible brain structures that could support this new learning. However, whether extinction engages the same brain sites necessary for acquisition is not yet clear. This poses an overriding problem for understanding brain mechanisms necessary for extinction because such analysis cannot be done without first identifying brain sites and pathways involved in this phenomenon. Equally elusive is the validity of a behavioral theory of extinction that can account for the properties of extinction. In this study, we looked at the involvement of the interpositus and the red nucleus in extinction. Results show that, although inactivation of both nuclei blocks response expression, only inactivation of the interpositus has a detrimental effect on extinction. Moreover, this detrimental effect was completely removed when inactivation of the interpositus was paired with electrical stimulation of the red nucleus. These findings speak to the important role of cerebellar structures in the extinction of discrete motor responses and provide important insight as to the validity of a particular theory of extinction.

The Journal of Neuroscience, March 5, 2008 • 28(10):2651–2658

Competitive and Noncompetitive Odorant Interactions in the Early Neural Coding of Odorant Mixtures

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Most olfactory receptor neurons (ORNs) express a single type of olfactory receptor that is differentially sensitive to a wide variety of odorant molecules. The diversity of possible odorant-receptor interactions raises challenging problems for the coding of complex mixtures of many odorants, which make up the vast majority of real world odors. Pure competition, the simplest kind of interaction, arises when two or more agonists can bind to the main receptor site, which triggers receptor activation, although only one can be bound at a time. Noncompetitive effects may result from various mechanisms, including agonist binding to another site, which modifies the receptor properties at the main binding site. Here, we investigated the electrophysiological responses of rat ORNs *in vivo* to odorant agonists and their binary mixtures and interpreted them in the framework of a quantitative model of competitive interaction between odorants. We found that this model accounts for all concentration-response curves obtained with single odorants and for about half of those obtained with binary mixtures. In the other half, the shifts of curves along the concentration axis and the changes of maximal responses with respect to model predictions, indicate that noncompetitive interactions occur and can modulate olfactory receptors. We conclude that, because of their high frequency, the noncompetitive interactions play a major role in the neural coding of natural odorant mixtures. This finding implies that the CNS activity caused by mixtures will not be easily analyzed into components, and that mixture responses will be difficult to generalize across concentration.

The Journal of Neuroscience, March 5, 2008 • 28(10):2659–2666

Neural Dissociation between Visual Awareness and Spatial Attention

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To what extent does what we consciously see depend on where we attend to? Psychologists have long stressed the tight relationship between visual awareness and spatial attention at the behavioral level. However, the amount of overlap between their neural correlates remains a matter of debate. We recorded magnetoencephalographic signals while human subjects attended toward or away from faint stimuli that were reported as consciously seen only half of the time. Visually identical stimuli could thus be attended or not and consciously seen or not. Although attended stimuli were consciously seen slightly more often than unattended ones, the factorial analysis of stimulus-induced oscillatory brain activity revealed distinct and independent neural correlates of visual awareness and spatial attention at different frequencies in the gamma range (30–150 Hz). Whether attended or not, consciously seen stimuli induced increased mid-frequency gamma-band activity over the contralateral visual cortex, whereas spatial attention modulated high-frequency gamma-band activity in response to both consciously seen and unseen stimuli. A parametric analysis of the data at the

single-trial level confirmed that the awareness-related mid-frequency activity drove the seen–unseen decision but also revealed a small influence of the attention-related high-frequency activity on the decision. These results suggest that subjective visual experience is shaped by the cumulative contribution of two processes operating independently at the neural level, one reflecting visual awareness per se and the other reflecting spatial attention.

The Journal of Neuroscience, March 5, 2008 • 28(10):2667–2679

NEUROBIOLOGY OF DISEASE

Neurodegeneration and Motor Dysfunction in a Conditional Model of Parkinson's Disease

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α -Synuclein (α -syn) has been implicated in the pathogenesis of many neurodegenerative disorders, including Parkinson's disease. These disorders are characterized by various neurological and psychiatric symptoms based on progressive neuropathological alterations. Whether the neurodegenerative process might be halted or even reversed is presently unknown. Therefore, conditional mouse models are powerful tools to analyze the relationship between transgene expression and progression of the disease. To explore whether α -syn solely originates and further incites these alterations, we generated conditional mouse models by using the tet-regulatable system. Mice expressing high levels of human wild-type α -syn in midbrain and forebrain regions developed nigral and hippocampal neuropathology, including reduced neurogenesis and neurodegeneration in absence of fibrillary inclusions, leading to cognitive impairment and progressive motor decline. Turning off transgene expression in symptomatic mice halted progression but did not reverse the symptoms. Thus, our data suggest that approaches targeting α -syn-induced pathological pathways might be of benefit rather in early disease stages. Furthermore, α -syn-associated cytotoxicity is independent of filamentous inclusion body formation in our conditional mouse model.

The Journal of Neuroscience, March 5, 2008 • 28(10):2471–2484

Subunit-Specific Trafficking of GABA_A Receptors during Status Epilepticus

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It is proposed that a reduced surface expression of GABA_A receptors (GABARs) contributes to the pathogenesis of status epilepticus (SE), a condition characterized by prolonged seizures. This hypothesis was based on the finding that prolonged epileptiform bursting (repetitive bursts of prolonged depolarizations with superimposed action potentials) in cultures of dissociated hippocampal pyramidal neurons (dissociated cultures) results in the increased intracellular accumulation of GABARs. However, it is not known whether this rapid modification in the surface-expressed GABAR pool results from selective, subunit-dependent or nonselective, subunit-independent internalization of GABARs. In hippocampal slices obtained from animals undergoing prolonged SE (SE-treated slices), we found that the surface expression of the GABAR β 2/3 and γ 2 subunits was reduced, whereas that of the δ subunit was not. Complementary electrophysiological recordings from dentate granule cells in SE-treated slices demonstrated a reduction in GABAR-mediated synaptic inhibition, but not tonic inhibition. A reduction in the surface expression of the γ 2 subunit, but not the δ subunit was also observed in dissociated cultures and organotypic hippocampal slice cultures when incubated in an elevated KCl external medium or an elevated KCl external medium supplemented with NMDA, respectively. Additional studies demonstrated that the reduction in the surface expression of the γ 2 subunit was independent of direct ligand binding of the GABAR. These findings demonstrate that the regulation of surface-expressed GABAR pool during SE is subunit-specific and occurs independent of ligand binding. The differential modulation of the surface expression of GABARs during SE has potential implications for the treatment of this neurological emergency.

The Journal of Neuroscience, March 5, 2008 • 28(10):2527–2538

Synergistic Neuroprotective Effects of Lithium and Valproic Acid or Other Histone Deacetylase Inhibitors in Neurons: Roles of Glycogen Synthase Kinase-3 Inhibition

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Lithium and valproic acid (VPA) are two primary drugs used to treat bipolar mood disorder and have frequently been used in combination to treat bipolar patients resistant to monotherapy with either drug. Lithium, a glycogen synthase kinase-3 (GSK-3) inhibitor, and VPA, a histone deacetylase (HDAC) inhibitor, have neuroprotective effects. The present study was undertaken to demonstrate synergistic neuroprotective effects when both drugs were coadministered. Pretreatment of aging cerebellar granule cells with lithium or VPA alone provided little or no neuroprotection against glutamate-induced cell death. However, copresence of both drugs resulted in complete blockade of glutamate excitotoxicity. Combined treatment with lithium and VPA potentiated serine phosphorylation of GSK-3 α and β isoforms and inhibition of GSK-3 enzyme activity. Transfection with GSK-3 α small interfering RNA (siRNA) and/or GSK-3 β siRNA mimicked the ability of lithium to induce synergistic protection with VPA. HDAC1 siRNA or other HDAC inhibitors (phenylbutyrate, sodium butyrate or trichostatin A) also caused synergistic neuroprotection together with lithium. Moreover, combination of lithium and HDAC inhibitors potentiated β -catenin-dependent, *Lef/Tcf*-mediated transcriptional activity. An additive increase in GSK-3 serine phosphorylation was also observed in mice chronically treated with lithium and VPA. Together, for the first time, our results demonstrate synergistic neuroprotective effects of lithium and HDAC inhibitors and suggest that GSK-3 inhibition is a likely molecular target for the synergistic neuroprotection. Our results may have implications for the combined use of lithium and VPA in treating bipolar disorder. Additionally, combined use of both drugs may be warranted for clinical trials to treat glutamate-related neurodegenerative diseases.

The Journal of Neuroscience, March 5, 2008 • 28(10):2576–2588