

2009

DEPARTMENT OF
Neuroscience

and the NEUROSCIENCE TRAINING AREA



photo by Patrick Hof

FIRST ANNUAL NEUROSCIENCE RETREAT

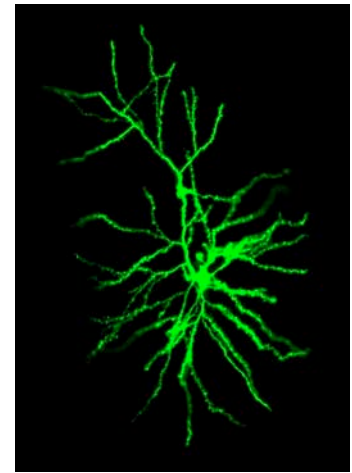
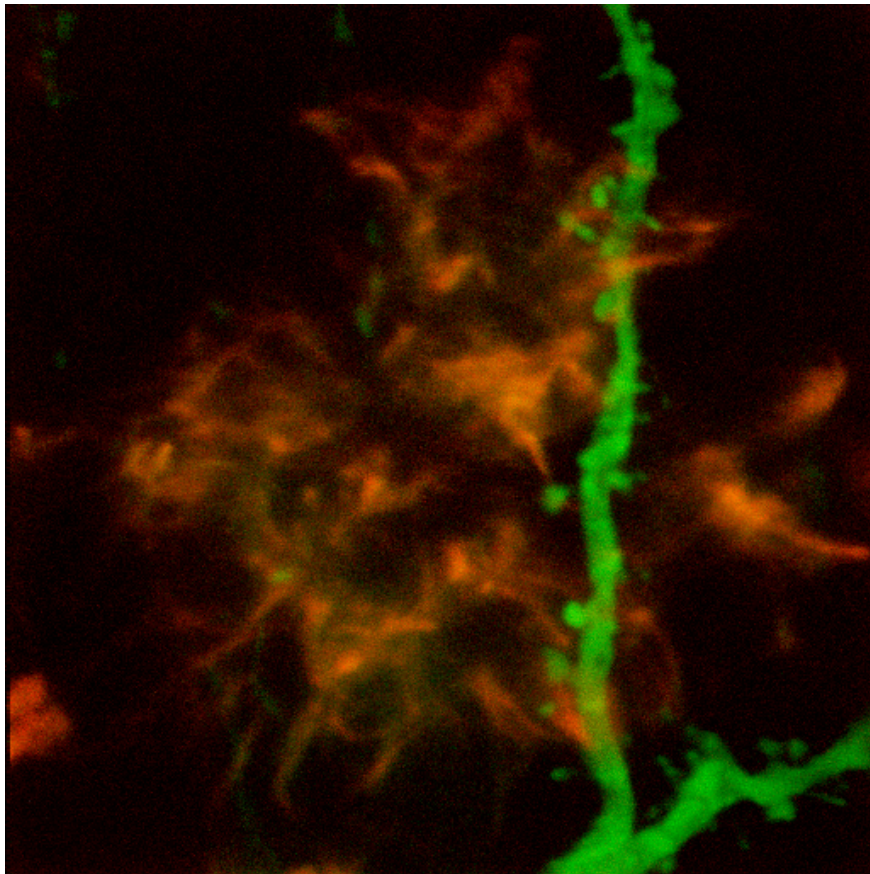


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Neuroscience Retreat Schedule

HOUSE OF THE REDEEMER 7 East 95th Street (btw Madison & Fifth)

10:00am.....Eric Nestler
10:25am.....George Huntley/Stephen Salton (Graduate Program)

SESSION 1- CHAIR (CHARLES MOBBS)

10:45am..... Emeline L. Maillet
11:05am..... Ektor. I. Manerakis
11:25am..... Samira Fargali
11:45am..... Camilla Butti

LUNCH

SESSION 2- CHAIR (YASMIN HURD)

1:30pm..... Shekhar Patil
1:50pm..... Jin Young Kim
2:10pm..... Ian Maze
2:30pm..... Dillon Y. Chen
2:50pm..... Tonya R. Anderson

ATRIUM-MOUNT SINAI

3:30pm.....Poster Session Begins

STUDENT LOUNGE (First Floor Annenberg Bldg, SE Corner)

4:30pm.....Reception Begins/Best Poster Award

Abstracts

1 Inhibition of T1R3 chemosensory receptor by the plant defensin Gurmarin peptide

Emeline L. Maillet, Laura Pelletier, Timothy J. Cardozo, Jeniffer Quijada, Marianna Max, Robert F. Margolskee.

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Gurmarin is a polypeptide of 35 amino-acids that suppresses behavioral and gustatory neural responses of rodents to sweet compounds without affecting responses to salty, sour, or bitter substances. Gurmarin has no detectable effect in human psychophysical studies. Here, we show that gurmarin acts on mouse T1R3 to antagonize the heterologously expressed mouse sweet receptor's response to a panel of sweeteners. Studies with human-mouse chimeras of T1R3 indicated that the first 150 amino acids (aa) of T1R3 must be from mouse to maintain sensitivity to gurmarin. Additional chimeras narrowed the region of importance to aa 40-80 of mT1R3. Based on the crystal structure of metabotropic class C GPCR glutamate receptor mGluR1, we created a homology model of the extracellular Venus Fly Trap Module of mT1R3. According to this model a 41-66 aa loop (loop1) is present within the upper lobe of the receptor's cleft. In our model, gurmarin docks to the receptor within the open cleft of the VFTM, directly in contact with the upper lobe. The difference in the 3D shape of loop1 of human T1R3 suggests that steric hindrance prevents gurmarin from binding to human T1R3's VFTM cleft. We hypothesize that gurmarin may inhibit activation of the sweet receptor by preventing proper adoption of T1R3 VFTM close active state. In addition, analysis of the interactions in the docked model between gurmarin and the receptor accord with previous work identifying key aromatic residues necessary for gurmarin function. Finally, point mutants altered at residues of mouse T1R3 predicted to interact with gurmarin displayed reduced sensitivity to gurmarin in in-vitro assays. Understanding the mechanism of gurmarin interaction may facilitate the development of peptide inhibitors of human T1R3, a target receptor with a potential therapeutical interest.

ELM was supported by NIH grants DC007984, DC006696, DC008301.

2 Looking for the nutritional “switch”: CtBP as the cellular transducer of the glucose derived nutritional signal

Ektor. I. Manerakis¹, Charles V. Mobss¹ 2009

Departments of Neurobiology and Geriatrics, Mt. Sinai School of Medicine, New York City.

The molecular basis of acquired obesity is largely unknown. Based on evidence that acquired obesity in mammals may entail impaired hypothalamic glucose sensing, we have developed a model of acquired obesity in *C.elegans*. We have observed that pharmacological inhibition of glucose sensing by 2-Deoxy-D-glucose, which induces obese phenotypes in mammals, induces obesity in *C.elegans*. We have further investigated the hypothesis that nuclear NADH, as a unique product of glucose metabolism, may play the role of a nutritional signal to couple nutrient availability, in the form of glucose, to cell metabolism and energy homeostasis by alterations in the cellular and nuclear output. We hypothesize that the COOH-terminal binding protein CtBP, a transcriptional suppressor, is responsible for conveying that NADH signal to the nucleus and producing the metabolic effects of glucose. We have shown that RNAi against the worm *ctbp-1* (homolog of mammalian CtBP) induced obesity in wild type worms. Furthermore, a screen for CtBP interacting proteins that might be implicated in the effect observed here identified *klf-1* as a factor necessary for the obese phenotype following *ctbp-1* silencing by RNAi.

3 **Analysis of Knockout Mice Suggests a Role for VGF in the Control of Fat Storage and Energy Expenditure**

Samira Fargali, Elizabeth Watson, Haruka Okamoto, Tandra Chakraborty, Mark W. Sleeman, Stephen R. Salton

VGF (non-acronymic) is a neurotrophin-regulated protein that is cleaved into bioactive peptides and secreted. VGF is expressed throughout the brain, in neurons, and in several neuroendocrine and endocrine tissues. Previous studies demonstrated that targeted deletion of *Vgf* on a mixed background produces extremely lean mice, that are hypermetabolic and resistant to diet-induced and genetically-induced obesity. The aim of this study was to clarify the role and the potential mechanism(s) and site(s) of action of VGF by analyzing the metabolic phenotypes of two independent VGF knockout (KO) lines on C57BI6 backgrounds. Both VGF KO lines had abnormal energy balance with elevated energy expenditure and inappropriate (i.e. normal) food intake for their increased metabolic rate. Compared with wild type, VGF KO mice had significantly less adipose tissue; MetaMorph was used to quantify morphological alterations in white adipose tissue (WAT) and brown adipose tissue (BAT). Standard formalin fixed slides, stained with hematoxylin and eosin, were used for morphometry analysis. There was a 39.2% decrease in the mean size of adipocytes in KO WAT compared with control WAT. We suggest, that this increased number of small adipocytes in VGF KO WAT may be due to adipocyte trans-differentiation towards a brown adipocyte phenotype, increased lipolysis and/or decreased lipogenesis. In addition, lipid droplet size in BAT was significantly decreased by 57.7% in KO compared with WT mice, which was also confirmed by electron microscopy. Levels of uncoupling protein-1 (UCP1), the primary regulator of thermogenesis, in BAT from VGF KO mice were significantly higher than WT, which correlated with an increase in the number of mitochondria per area (74.3% higher than WT) by EM. Our studies suggest that increased mitochondrial number with more densely packed cristae and elevated UCP1 levels are responsible for increased energy expenditure and leanness in VGF-deficient mice. The mechanisms by which VGF and/or VGF-derived peptides regulate fat storage and energy expenditure, and the other proteins involved in fatty acid oxidation and lipid storage in BAT and WAT, are currently under investigation.

4 **Von Economo neurons in the cerebral cortex of cetaceans: distribution and quantification**

*C. BUTTI^{1,2}, B. WICINSKI¹, J. S. REIDENBERG³ and P. R. HOF¹

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Von Economo neurons (VENs) are a class of bipolar and large-sized projection neurons thought to play a crucial role in the neural circuitry responsible for social awareness and assessment of complex social situations. The loss, morphological alteration or abnormal development and distribution of VENs in humans are known to be related to a wide spectrum of neuropsychiatric conditions such as frontotemporal dementia, schizophrenia and autism. VENs have been described in layer V of the anterior insular and anterior cingulate cortices of humans, great apes, and cetaceans and in layer V of the dorsolateral prefrontal and frontopolar cortices of humans and cetaceans. The selective distribution of VENs in the cetacean brain is intriguing and consistent with the growing evidence of their sophisticated cognitive abilities and social lifestyles such as complex social structures, long-term bonds, higher-order alliances, cooperative networks, cultural transmission, tool use, and mirror self-recognition. In an attempt to understand the neuroanatomical basis of cognition in cetaceans we used a stereological approach to obtain volume estimates and to perform an exhaustive count of the total number of VENs in the anterior cingulate, anterior insular and frontopolar cortices of representative taxa of the order Cetacea such as the bottlenose dolphin (*Tursiops truncatus*), the Risso's dolphin (*Grampus griseus*), the pilot whale (*Globicephala melas*), the beluga whale (*Delphinapterus leucas*), the humpback whale (*Megaptera novaeangliae*), and the minke whale (*Balaenoptera acutorostrata*). Our results show that the distribution, the size and the total number of VENs in the cetacean brain is comparable to what has been previously described in great apes, with VENs being mainly concentrated in the crown of the gyri and having a larger volume than neighboring layer V pyramidal cell and fusiform neurons of layer VI. The presence of VENs in groups of mammals with complex social structures and greatly divergent evolutionary histories, such as humans, great apes, and cetaceans, indicates that VENs evolved at least twice and may represent an obligatory evolutionary outcome to ensure fast transmission of information in circuits responsible for higher-level cognitive processes in very large brains.

5 Neuropathic pain-induced regulation of cannabinoid CB₁ receptors in higher centers of the CNS.

Shekhar Patil¹, Ittai Bushlin², Lakshmi A. Devi², and George W. Huntley¹

¹Dept. of Neuroscience and ²Dept. of Pharmacology and Systems Therapeutics, Mount Sinai School of Medicine, New York, NY 10029

Chronic neuropathic pain has both sensory and affective behavioral components that impact mood, motivation and motor control. Both components are affected significantly by opioids and cannabinoids, compounds that have analgesic and addictive properties. Recent attention has focused on the role of peripheral and spinal cannabinoid receptors, which are largely localized on presynaptic axons, in modulating pain and analgesia. However, cannabinoid receptors (CBRs), particularly the CB₁R subtype, are widely expressed throughout higher centers of the CNS, but it is unclear whether these receptors play a role in pain-related experiences. Using a combination of behavioral and immunolocalization methods applied to a rat L5 spinal nerve lesion (SNL) model of neuropathic pain, we show here that CB₁R immunofluorescence is rapidly diminished in the external globus pallidus (GPe) on the contralateral side following unilateral L5 SNL. Such loss is not accompanied by changes in striatal or pallidal immunolocalization of GAD, a marker of GABAergic input to GPe, nor are there changes in CB₁R in other CNS structures examined, e.g., substantia nigra. Treating lesioned-rats with an escalating daily dose of morphine for 5 days diminished pain sensation and blocked the pain-associated changes in CB₁R. Taken together, these data indicate pain-associated interactions between opioid and cannabinoid receptor systems in higher CNS structures that likely influence perception of pain and/or pain-related changes in affective or motor behaviors. The data underscore shared neurochemical/anatomical substrates underlying pain, analgesia, and drugs of abuse.

Supported by DA08863 (to LAD).

6 Inducible nuclear HDAC1 export is essential for the early signs of axonal damage

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Histone deacetylase 1 (HDAC1) is a well characterized nuclear enzyme. We demonstrate here that in response to pathological stimuli, HDAC1 is exported from the nucleus to the cytoplasm of neurons, where it induces morphological and functional changes. The cytosolic localization of HDAC1 is preceded by binding to the nuclear receptor CRM-1 and followed by localized neuritic swellings. The onset of swellings could be prevented either by silencing HDAC1 using shRNA lentiviral particles and Cre-lox system or by inhibition of CRM-1 dependent nuclear export using a pharmacological inhibitor, leptomycin B. Over-expression of a mutant HDAC1 that constitutively accumulates in the cytosol, is sufficient to initiate neuritic swelling even in the absence of toxic stimuli. MALDI-TOF identification of HDAC1-binding proteins in treated neurons and in extracts from demyelinated brain regions reveals axonal motor proteins and cytoskeletal components. These proteins interact with HDAC1, but not with other isoforms, only in response to pathological conditions, thereby suggesting a previously unrecognized role for nucleo-cytoplasmic export of HDAC1 in the early events leading to axonal damage.

Supported by grants from the NJ Commission Brain Trauma (07-3203-BIR-E-0), from National Multiple Sclerosis Society (RG3957), from NIH (RO1-NS42925) and funds from the Multiple Sclerosis Research Foundation

7 Essential role of the histone methyltransferase G9a in cocaine-induced plasticity

Ian Maze¹, Herbert E. Covington, III¹, David M. Dietz¹, Quincey Laplant^{1,2}, William Renthal², Scott J. Russo¹, Max Mechanic², Ezekiel Mouzon¹, Rachael L. Neve³, Stephen J. Haggarty^{4,5}, Paul Greengard⁶, Alexander Tarakhovsky⁷, Anne Schaefer⁶, and Eric J. Nestler¹

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Chronic cocaine-induced alterations in gene expression cause changes in neuronal morphology and behavior that are thought to underlie cocaine addiction. Here, we identify an essential role for histone 3 lysine 9 (H3K9) methylation and the lysine methyltransferase G9a in cocaine-induced structural and behavioral plasticity. We demonstrate that chronic, but not acute, cocaine administration reduces global levels of H3K9 methylation in the nucleus accumbens, a key brain reward region. This reduction in histone methylation is mediated through the downregulation of G9a in this brain region, which in turn is regulated by the cocaine-induced transcription factor, Δ FosB. Using conditional mutagenesis and viral-mediated gene transfer, we demonstrate that G9a downregulation mediates increased dendritic arborization of nucleus accumbens neurons and enhanced cocaine reward, thereby establishing a crucial role for histone methylation in the long-term actions of cocaine.

8 Molecular Convergence Between the Glucocorticoid Receptor- and BDNF-dependent Pathways During Long-Term Memory Consolidation.

Dillon Y Chen, Dhananjay Bambah-Mukku, Gabriella Pollonini, Cristina M Alberini

Department of Neuroscience, Mount Sinai School of Medicine, New York, NY. 10029

Newly learned information becomes long-term memory through a process known as consolidation, which depends on an initial phase of transcription and translation. A wealth of literature suggests that *de novo* protein synthesis and the evolutionarily conserved CREB-C/EBP (cAMP Response Element Binding protein – CCAAT/Enhancer Binding Protein) are critically required for the formation of long-term memory. However, the upstream signaling pathways and the downstream effectors that are linked to the induction of protein synthesis and activation of the CREB-C/EBP pathway *in vivo* during memory consolidation remain to be elucidated. We found that the BDNF and the glucocorticoid receptor (GR) signaling pathways are major contributors of the activation of the molecular cascades underlying memory consolidation in the hippocampus. Using inhibitory avoidance, we found that blockade of either the BDNF or the GR signaling pathway in the hippocampus mimics the effects of inhibiting hippocampal protein synthesis on long-term memory consolidation. Similar temporal effects on memory retention are seen when protein synthesis, BDNF or GR is blocked; specifically, blockade of either hippocampal protein synthesis, BDNF or GR before training resulted in significant disruption of memory if compared to the same treatment given immediately after training. Furthermore, blocking either the BDNF or the GR pathway does not affect short-term memory, a process that does not require protein synthesis. In addition, the amnesia caused by inhibition of GR is rescued by co-administration of BDNF. We are testing the hypothesis that a cross-talk between GR and BDNF significantly contributes to the activation of the protein synthesis, CREB-C/EBP-dependent cascade underlying long-term memory consolidation.

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Hirschl

9 Bidirectional AMPA Receptor Trafficking during NMDA Receptor-Dependent Hippocampal Plasticity: The Role of N-Cadherin

Tonya R Anderson and Deanna L. Benson

Trafficking of AMPA receptors into and out of synapses is thought to largely account for changes in synaptic efficacy during long-term potentiation and depression, but how this trafficking is coupled to differential NMDA receptor activation is a longstanding question. We find that this coupling is achieved via the calcium-dependent cell adhesion molecule N-cadherin, which is present in complex with AMPA receptors and whose homophilic adhesion is modulated by NMDA receptor activity. Blocking N-cadherin adhesion in mature hippocampal cultures during or after glycine induction of synaptic potentiation blocks the subsequent increase in synaptic AMPA receptor content normally generated. Moreover, when N-cadherin has been knocked down by RNA interference, AMPA receptors are neither gained after glycine stimulation nor lost following a glycine-based protocol for long-term depression that reduces AMPA receptor content in control neurons. Notably, baseline AMPA receptor levels are reduced following knockdown, suggesting a chronic role for N-cadherin in AMPA receptor trafficking during synaptogenesis. Taken together, these findings suggest that N-cadherin based adhesion confers accumulation and/or retention of AMPA receptors to sites of NMDA receptor activation.

10 Effects of hormone replacement regimens on morphological markers of plasticity in young primate CA1.

Megan Bailey, PI John Morrison

The study of changes in cognitive function in an aging population is a major and growing focus of neuroscience research. In pursuing this study, of major value are compounds that can be demonstrated to affect measures of cognitive function in addition to effecting changes in the structure or function of areas of the brain associated with learning and memory. Estrogen is one particularly interesting such compound, as levels of estrogen drop off precipitously in women as they go through menopause in middle age, after which age-related cognitive impairment is most likely to present. The interaction between this loss of estrogen and the decline in cognitive function experienced by many aging individuals is not well known, and, though studies in rodent and primate models show predictable and significant benefits of estrogen therapy regimens on both cognitive performance and morphologic and molecular markers of hippocampal and prefrontal cortical plasticity, studies designed to explore the cognitive benefits of estrogen therapy in human women have yielded conflicting results. Studies in nonhuman primates, which have menstrual cycles which are fundamentally similar to their human counterparts in duration and character, are thus important as the next step to tease out specific parts of the mechanism of action of estrogen replacement therapies in order to determine the time window and treatment course which should be pursued in a clinical setting for maximum cognitive benefit.

In rat and nonhuman primate models, high physiological or pharmacologically induced estrogen levels raise spine density in the CA1 subfield of the hippocampus. This increase in spine density occurs through a preferential increase in the number of small thin spines. These small spines are rich in NMDA receptors and are very motile. It has been suggested that this population represents a pool of spines which are available to support the formation of new memories, and as such that the size of this pool may be a marker of plasticity in an area of the cortex. The current study measures both postsynaptic density length (as a measure of spine size) and synapse density in CA1 in twenty young ovariectomized (OVX) monkeys given a variety of hormone regimens designed to compare the state of the hippocampus at the trough of the estrogen cycle in a cyclic regimen with the effects of continuous estrogen therapy. The data suggest that animals in the cyclic trough are identical to vehicle animals over these two measures, while continuous estrogen-treated animals show a trend towards higher synapse density and a larger population of the smallest spines. Ongoing research in the dlPFC of these animals should clarify these results.

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Multiple functions of ezrin in the development of mouse neocortex

Ioana Carcea, Deanna L Benson

The development of neurons in the mammalian neocortex succeeds in a precisely controlled series of events: progenitor proliferation in the ventricular zone, neuronal differentiation, neuron migration in the cortical plate and extension of axons and dendrites toward appropriate targets. The molecular mechanisms controlling these events are poorly understood. Human and mouse genetics point to a role for L1 cell adhesion molecule in these processes, but how signals are transmitted from this transmembrane molecule to intracellular effectors is unknown. Our lab has previously identified a direct interaction between the cytoplasmic tail of L1 and ezrin. Ezrin is a membrane-cytoskeleton linker expressed by a variety of tissues, where it regulates the acquisition of cell-type specific morphological features as well as cell migration, polarization and proliferation. Ezrin transcripts and proteins are detected in the developing neocortex, most abundantly in the ventricular zone and the cortical plate. Dissociated cortical neurons express the protein and its active, phosphorylated, form in cell bodies and in axonal and dendritic filopodia. We asked what role ezrin plays in the development of layer 2/3 cortical neurons *in vivo*. To address this, we introduced either a dominant negative or a constitutively active ezrin in layer 2/3 neurons by *in utero* electroporation at embryonic day 15. The dominant negative ezrin alters neuron morphology and delays neuron migration in the cortical plate. Constitutively active ezrin has only modest effects on migration, but dramatically decreases the survival of neurons in the cortical plate. We show that in addition to L1, ezrin activity is responsive to Sema3A, Netrin-1 and BDNF, well known secreted molecules that contribute to both neuronal migration and survival, and we will address whether ezrin-mediated effects on migration and survival are downstream from these cues.

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12 Role of CCAAT enhancer binding protein delta (C/EBP δ) in memory consolidation.

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The CCAAT enhancer binding protein (C/EBP) family of transcription factors has been shown to play a role in learning and memory. We have previously reported that, in rat, the transcription of both C/EBP beta (C/EBP β) and delta (C/EBP δ) is induced in the hippocampus after the acquisition of inhibitory avoidance (IA) and that C/EBP β plays an essential role in the hippocampus during memory consolidation and in the amygdala during memory reconsolidation. A global knockout of C/EBP δ in mice results in an enhancement of contextual and auditory fear memory, but does not affect the retention of water maze memory; however, the role of C/EBP δ in specific brain regions on memory consolidation remains to be determined. Toward this end, Long Evans rats were trained in the IA task and both the expression levels of C/EBP δ and its functional role in the dorsal hippocampus and basolateral amygdala were investigated. Quantitative western blot analyses revealed that protein levels of CEBP δ are unchanged 1 hour after training, but there is a robust induction 20 hours after IA training. Furthermore, knock-down of the hippocampal expression of C/EBP δ via bilateral oligodeoxynucleotide (ODN) injections 5 or 12 hrs after acquisition of IA either into the basolateral amygdala or dorsal hippocampus result in amnesia 48 hrs after training. Hence, C/EBP δ is essential in both regions for IA memory consolidation. Interestingly, immunohistochemistry, western blot and electromobility shift assay show that C/EBP δ is present in both the nucleus as well as dendrites of hippocampal and cortical neurons. Ongoing studies aim at determining the regulation and functional role of C/EBP δ in both nuclear and dendritic compartments.

13 Astrocytes play a critical role in hippocampal long-term memory formationAkinobu Suzuki¹, Sarah Stern¹, Dhananjay Bambah-mukku¹, Pierre Magistretti² and Cristina M. Alberini¹¹Department of Neuroscience, Mount Sinai School of Medicine, New York, NY. 10029²Laboratory of Neuroenergetics and Cellular Dynamics, Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne, Lausanne (EPFL), CH-1015 Switzerland

The neurometabolic coupling between synaptic activity and glucose utilization is central for the physiology of brain functions. Glycogen, which is primarily stored in astrocytes, is believed to provide an energy source for the astrocytes themselves and/or for surrounding neurons. Previous results in chicks have shown that inhibition of glycogenolysis using the glycogen phosphorylase inhibitor, 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) disrupts bead discrimination memory consolidation. To determine the role of astrocytic functions in hippocampus-dependent memory formation, we injected DAB bilaterally into the hippocampus of rat at specific time points before or after inhibitory avoidance (IA) training. DAB injected 15 minutes before or immediately after training significantly blocked long-term memory (24h) in a dose –dependent manner, while leaving short-term memory (1h) intact, indicating that astrocytic glycogen metabolism is required for memory consolidation in the hippocampus. In the neurometabolic coupling model, the basic mechanisms include glutamate-stimulated aerobic glycolysis, the sodium-coupled reuptake of glutamate by astrocytes and the ensuing activation of the Na-K-ATPase that triggers glucose uptake and processing via glycolysis, resulting in the release of lactate from astrocytes. Lactate can then contribute to the activity-dependent neuronal energy demands associated with synaptic transmission. To determine whether the effect we found with DAB was due to the astrocytic release of lactate, we tested the effect of antisense-mediated disruption of the monocarboxylate transporter 1 (MCT1), which is known to selectively regulate the release of lactate from astrocytes. Consistent with the results using DAB, injection of antisense oligodeoxynucleotides specific for MCT1 into the hippocampus 1 hour before training completely and persistently blocked memory consolidation. Taken together, these findings indicate that astrocytic glycogenolysis and release of lactate play an essential role in memory formation in the hippocampus.

14 Function and mechanisms of memory reconsolidation

Inda MC, Muravieva E, Riceberg J, Alberini CM

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Newly learned information is initially in a labile state and over time stabilizes into a long-term memory by a consolidation process known to require RNA and protein synthesis. Established memories can again become transiently sensitive to disruption if reactivated, and similar to a newly formed memory, require a process of reconsolidation in order to be maintained. The function/s of reconsolidation still remain to be elucidated. Here, we tested the hypothesis that reconsolidation mediates memory strengthening and prevents forgetting. We found that multiple reactivations lead to a significant increase in the retention of inhibitory avoidance (IA) memory. This increase can be disrupted if protein synthesis is inhibited after each reactivation. Importantly, the increase in memory strength occurs only when the memories are recent. Furthermore, multiple reactivations, in addition to increasing memory strength, lead to an increased resistance to disruption by protein synthesis inhibitors. The sensitivity to disruption of the memory after reactivation also depends on the duration of the reactivating event. Post-reactivation inhibition of protein synthesis significantly disrupts memory reconsolidation only when the reactivation session is relatively long lasting (e.g. 90 seconds, full testing), but has no significant effect if the reactivation event is brief (10 seconds). However, this memory is disrupted if protein synthesis is inhibited before the reactivation event, suggesting that a fast synthesis of proteins mediates memory reconsolidation and strengthening.

This work was supported by R01 MH074736 and Human Frontier Science Program

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Role of ERK in mechanisms of actions of hallucinogens

Ang RL, González-Maeso J, Gingrich JA and Sealfon SC.

Hallucinogens are psychoactive substances that alter perception, mood, and a host of cognitive processes. Although serotonin 2A (HTR2A), a G protein coupled receptor, has been established as the key receptor involved in their mechanisms of actions, the signaling processes involved and how hallucinogens generate their specific neuropsychological responses are poorly understood. We have found hallucinogens act at HTR2As via Gi/o to specifically up-regulate *egr-1* and *egr-2* in mouse cortical neurons. In contrast, *c-fos* is induced by all HTR2A agonists through the Gq mediated phospholipase C cascade. These results implicate agonist-directed signal trafficking and activation of the two main HTR2A mediated signaling cascades in the effects of hallucinogens. We also reported that HTR2A and metabotropic glutamate 2 receptor (Grm2) are heterodimers in vitro and act together to mediate the signaling responses of hallucinogens in vivo. Both Gi/o and Gq signaling can activate the ERK cascade to elicit the differential neuronal gene response. It has been speculated that ERK activation may act as a “gate” with dual function in neuroplasticity and behavioral changes. In the present study, we have investigated the role of ERK1/2 in hallucinogen-specific signaling as well as its role in eliciting the robust acute 2AR-mediated behavioral response of hallucinogen - head twitch response in murine model. Using SL327, a chemical inhibitor of ERK signaling that crosses blood brain barrier, the *egr-1* and *egr2* up-regulation studied in vivo and primary cortical neuronal cells was ablated and the head twitch response was significantly reduced, suggesting ERK activation is a key component in the mechanisms of actions of hallucinogens. Using LY379268 (Grm2 and metabotropic glutamate 3 receptor agonist), we are determining the role HTR2A-Grm2 dimers in mediating the activation of ERK response. We find an altered compartmentalization of phospho-ERK in primary cortical neuronal cells, elicited by 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI), a hallucinogen, in the presence and absence of LY379268. These results suggest a novel mode of mechanism for control of the cellular and behavioral responses elicited via a G-protein-coupled receptor.

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Glucose-induced neurotoxicity: Mechanisms and screen for protective drugs.

Fumiko Isoda and Charles Mobbs

Neuropathy is the most common complication of diabetes, affecting about 70 million Americans and constituting the major risk factor for diabetic amputations and chronic pain. Mechanisms mediating toxic effects of elevated glucose on neurons are not understood, and the only treatments for neuropathy address symptoms, not underlying pathophysiology. To develop a method to study mechanisms of diabetic neuropathy and a screen for drugs to prevent glucose-induced neuronal damage, we developed a high-throughput method to study this process. Since diabetic complications are thought to involve oxidative stress, we first we carried out a dose-response curve of hydrogen peroxide, in the presence of low and high glucose, to determine a threshold dose of hydrogen peroxide to produce neuronal death. The viability assay reduction of soluble tetrazolium salt which produces a water-soluble formazan dye soluble in the tissue culture medium in proportion to the number of living cells. We found that at 100 uM hydrogen peroxide, 15 mM glucose (diabetic levels) reduced viability by more than 50% compared to 5 mM glucose, whereas in the absence of hydrogen peroxide there was no difference in viability at the two glucose concentrations. With respect to mechanism, we observed that lactate mimicked the toxic effects of 15 mM glucose under these conditions, but pyruvate did not, implicating cytoplasmic NADH glucose-induced toxicity. With respect to protective compounds, we screened over 1700 compounds from a library of drugs approved for use in humans. The screen involved exposing neurons to H₂O₂ for one hour in the presence of 15 mM glucose, followed by exposure to the drug for 3 hours, followed by restoration of normal medium and assessment of viability 24 hours later. Assays were run in replicates of 8; when viability was increased by t-test, drugs were subsequently tested by a second measure of viability, release of lactate dehydrogenase. After the second set of assays, 30 compounds were observed to significantly enhance viability after glucose-induced toxicity. Several of these drugs have now been shown to be protective in a *C. elegans* model of Alzheimer's disease.

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“The APP Knockout Mouse as a Model of Alzheimer’s Disease”

Jessica Walsh, Dara L. Dickstein, PhD, and Patrick R. Hof, MD

The amyloid precursor protein (APP) plays an essential role in the pathogenesis of Alzheimer’s disease (AD). The processing of APP involves proteolytic cleavage by β - and γ -secretases, in order to produce the amyloid β protein (A β), which is the major protein component of the senile plaques in AD. α -secretase also cleaves APP, resulting in the release of the large soluble APP (sAPP) luminal domain. While the specific role of APP is still unclear, its potential functions include mediation of neurite outgrowth, cell adhesion, and regulation of synaptic plasticity and transmission. Studies have shown synaptic deficits and decrease in spines prior to the accumulation of plaques in AD and in APP transgenic mouse models, which attributed to A β toxicity. Furthermore, APP $^{-/-}$ mice have been shown to have deficits in cognitive and motor functions at 4 and 10 months including impairment in conditioned avoidance and Morris water maze tasks. In this study, we used APP $^{-/-}$ mice, which have a deletion of the promoter and first exon of the mouse APP gene, to assess possible changes in neuronal morphology to understand whether APP has an effect on dendritic integrity. We focused on brain regions associated with AD pathology, specifically CA1 and dentate gyrus (DG) in the hippocampus, and prefrontal cortex (PFC). Mice were perfused and intracellular injections of Lucifer Yellow were made in neurons in the CA1 and DG of the hippocampus, and in the PFC. Neurons were traced using NeuroLucida (MBF Bioscience) software and Sholl analysis was performed to quantify dendritic length and complexity. We found that compared to controls, CA1 neurons have shorter apical length and decreased number of intersections in aged APP $^{-/-}$ mice, but no significant differences in basal dendritic length or complexity. Furthermore, DG granule cells showed no significant difference in mean dendritic length and number of intersections beyond trends towards shorter dendritic lengths and fewer intersections close to the soma. PFC pyramidal neurons showed shorter apical length and decreased number of intersections in APP $^{-/-}$ mice, but no significant difference in basal dendritic length or complexity. These findings suggest that APP plays a key role in the formation and complexity of dendrites. Mice lacking the APP gene showed significant morphological alternations in dendrites of neurons located in CA1, DG and the PFC. The alterations in the apical dendrites in the absence of APP suggest that APP plays a role in synapse formation and that the APP-dependent component of A β may induce synaptic damage. Further studies will focus on the alterations in spine density and spine types in these animals.

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Characterization of muscle-specific tyrosine kinase receptor expression in the rat brain

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The muscle-specific tyrosine kinase receptor (MuSK) mediates the formation of neuromuscular junctions through the clustering of nicotinic acetylcholine receptors (nAChRs) found in muscle. Our laboratory has shown that MuSK is also present in the adult rat brain, and its expression in the hippocampus mediates memory consolidation suggesting a role for MuSK in long-term synaptic plasticity. In this study, we have used double-labeling immunohistochemistry combined with confocal microscopy to determine the cellular and subcellular distribution of MuSK in the brain and its expression pattern through development. We found the highest cortical expression of MuSK at embryonic day 18.5 (E18.5) which decreased postnatally to the levels observed in adult animals. MuSK is expressed robustly in the ventricular zone and the cortical plate of E18.5 rats and co-localizes extensively with the excitatory receptors nAChR, N-methyl D-aspartate receptor (NMDA-R), and the gamma-amino butyric acid receptor type A (GABA_A-R) suggesting MuSK is a post-synaptic protein. MuSK co-localization with the NMDA-R and nAChR decreases developmentally over time, but is still present in adult animals. Furthermore, in the adult brain MuSK does not co-localize with the pre-synaptic markers synapsin and synaptophysin, or with the post-synaptic marker MAP-2, although the presence of MuSK is visible in neuronal processes. Lastly, MuSK co-localizes extensively with the neurotrophic tyrosine kinase receptor B (TrkB) suggesting that MuSK might participate in synaptic plasticity events via neurotrophic-dependent pathways. Future studies will aim at testing the functional role of MuSK in the developing brain, as well as in synaptic structural changes in adult animals.

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Synaptic depression and the loss of the program-generating ability of interneuron B65 in Aplysia

Michael Due

In *Aplysia californica*, stimulation of interneuron B65 is sufficient to elicit egestive motor programs but with repeated B65 stimulation these programs decrement (Kabotyanski et al 1998). Here we show that, at first, stimulation of B65 generates biphasic programs consisting of protraction and retraction. When B65-elicited motor programs are repeatedly induced, the retraction phase ceases to occur. Continued induction of B65-elicited motor programs causes the protraction phase to either disappear or end prematurely. B65, which contains GABA and DA (Diaz-Rios et al 2002), makes fast excitatory connections with protraction interneurons/motoneurons within the central pattern generator (ex. B31/32, B61/62, and B63) whose functions are critical for the generation of both the protraction phase and the retraction phase (Kabotyanski et al 1998, Susswein et al 2002, Due et al 2004, Hurwitz et al 1997). During repetitive induction of B65-elicited motor programs the sustained depolarization of protraction neuron B31/32 is significantly reduced. Also, the firing frequency of protraction neurons B61/62 and B63 is significantly reduced, and the latency from the onset of B65 stimulation to the first action potential observed in B31/32, B61/62, and B63 is significantly increased. The fast one-for-one EPSPs from B65 to protraction neurons B31/32, B61/62 and B63 undergo dramatic synaptic depression following repetitive stimulation of B65 and take at least 20 minutes to recover. In view of the widespread decrement of B65-elicited EPSPs we probed the possibility that this plasticity has a presynaptic component. We imaged the neuropile area in which B65 processes overlap those of its followers. We compared the increase of Ca^{2+} concentration in response to a test B65 stimulation before and after repeated B65 stimulation. We found that the elevation of Ca^{2+} in response to test stimulation was smaller after repeated B65 stimulation. This decrease in B65 Ca^{2+} concentration persisted for at least 20 minutes. Thus, decreased Ca^{2+} concentration in B65 may contribute to the synaptic depression of B65-elicited EPSPs and B65's failure to generate motor programs that are observed following repeated B65 stimulation.

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Activity Regulated Local Protein Turnover In Cultured Hippocampal Neurons

Kuangfu Hsiao and Deanna L. Benson

Enduring modifications in synaptic efficacy require precise control over the profile of proteins expressed at individual synapses. Neurons face the formidable challenge of regulating protein content at specific subsets of synapses. One attractive solution to this problem is the local translation of mRNAs that are targeted to dendrites and perhaps to synapses, and activity mediated synaptic protein turnover. The molecular mechanisms mediating such local modifications of protein content are now being elucidated. We developed a fluorescence reporter system to visualize spatial dynamics of dendritic protein expression in cultured neurons. An mRNA dendritic localization signal from the Calcium/calmodulin-dependent protein kinase II mRNA 3' un-translated region was inserted behind the stop codon of a photoswitchable fluorescence reporter. Ratios between images taken at different wavelengths and comparisons between dendrites and cell bodies allow us to identify newly synthesized and pre-existing proteins in dendrites. A pleckstrin homology domain, which binds to membrane phosphatidylinositol lipids, was fused to the carboxyl terminus of the reporter to limit diffusion. We observed the rate of fluorescence accumulation in distal dendrites is approximately three-fold greater than the proximal area in cultured hippocampal neurons expressing our reporter. A control reporter lacking the dendritic targeting signal accumulates more rapidly in proximal dendrites and falls off in distal dendrites, as would be predicted by free mRNA diffusion. Thus, the increases observed with the targeted reporter are probably the result of active transportation mediated by the dendritic localization signal. When neurons were stimulated by exposure to high potassium, the rate of fluorescence accumulation changed immediately. At soma and proximal dendrites, the rate of reporter accumulation increased after stimulation; at distal dendrites, the rate decreased after stimulation. The control reporter increased uniformly throughout neurons. These data suggest three possibilities: 1) activity increases mRNA transport or, 2) activity represses translation of dendritic mRNAs at distal sites; 3) activity increases protein degradation at distal dendrites.

21 **Role of caloric source in neuropathology in Alzheimer's mouse model: Preliminary association of high protein diet with neuronal loss in hippocampal CA3**

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The causes of the onset and progression of Alzheimer's disease (AD) have yet to be elucidated. Recently, an increased interest in the role diet plays in the pathology of AD resulted in a focus on the detrimental effects of diets high in cholesterol and fat and the beneficial effects of caloric restriction. The current study examines how, in the face of standardized cholesterol, the source of calories modulates cerebral amyloidosis and neuronal integrity in the TgCRND8 mouse model of AD harboring three missense mutations in the APP gene (KM670/671NL and V717F). Mice were fed one of four diets: high fat (60%), high protein (60%), high carbohydrate (60%), or regular chow. Consistent with previous reports, we observed that animals receiving a high fat diet showed increased levels of both A β 40 and 42. Surprisingly, neuronal density and volume tended to decrease in the hippocampal CA3 region in TgCRND8 mice receiving the high protein diet, whereas CA1 and the dentate gyrus neuronal density and volume were preserved. The loss of CA3 neurons, whereas significant at $p < 0.05$ in an uncorrected ANOVA, did not withstand Bonferroni correction, hence indicating only a trend toward neuronal loss in the high protein fed TgCRND8 mice. With the caveat that larger follow-up sample sizes are required to confirm our observations, these data suggest that diet plays a role in the extent of neuronal death in this and other models of AD. Furthermore, these data raise the possibility that diet plays a role not only in modulating amyloidosis but also in modulating neuronal vulnerability to amyloid pathology.

22 **Chronic Cocaine Engages a Feedback Loop Involving Δ FosB and CaMKII in the Nucleus Accumbens**

A.J. Robison, Matt Wilkinson, Georgia Dolios, Said Kourrich, Mark Thomas, Rong Wang, and Eric Nestler

The transcription factor Δ FosB is stably induced in the nucleus accumbens (NAc) by chronic exposure to cocaine and mediates sensitized responses to cocaine exposure. We have previously demonstrated that phosphorylation of Δ FosB by casein kinase 2 at Ser27 regulates Δ FosB stability in vivo (Ulery et al., 2006 and 2009), but it is unknown whether other kinases can phosphorylate this protein. Calcium/calmodulin-dependent protein kinase II (CaMKII) is a neuronally-enriched serine/threonine protein kinase whose activity in the NAc is increased by drugs of abuse, including cocaine. Here, we demonstrate that Δ FosB is a potent substrate for CaMKII at multiple sites, including Ser27. Moreover, overexpression of constitutively active CaMKII in the mouse NAc regulates Δ FosB levels in vivo. Finally, we demonstrate that Δ FosB binds at multiple AP-1 consensus sites within CaMKII promoter regions and may regulate CaMKII mRNA levels in response to cocaine. In combination, these data suggest that CaMKII and Δ FosB may engage in a classic negative feedback loop as a mechanism for regulating reward circuitry in response to chronic cocaine administration.

23 Effects of HDAC inhibitors infused into specific brain areas implicated in depression after chronic social defeat stress in mice

Herbert E. Covington III, Ian S. Maze, Quincey C. LaPlant, Vincent F. Vialou, Olivier Berton, William Renthal, Vaishnav Krishnan, Scott J. Russo, Dan M. Fass, Stephen J. Haggarty, and Eric J. Nestler

Novel treatments for depression are urgently needed, and modifying chromatin, using histone deacetylase inhibitors, may have potential for providing relief from some depressive symptoms. In mice, chronic social defeat stress engenders persistent depressive-like behaviors and alters histone acetylation in limbic structures, including the nucleus accumbens. While several brain regions are implicated in the expression of depressive-like behaviors, we have examined the precise role of increasing histone acetylation within the nucleus accumbens, hippocampus, amygdala, and prefrontal cortex. Specifically, we investigated the influence of HDAC inhibitors infused directly into each of these brain areas subsequent to ten days of social defeat stress. Continuous bilateral infusion of the HDAC inhibitors SAHA or MS-275 via osmotic mini-pumps was initiated immediately after the last day of social stress. Over the next 14 days of HDAC inhibitor delivery, defeated and non-defeated control mice were examined across three behavioral assays: social interaction, sucrose preference, and forced swim task. Chronic social defeat stress reliably induced depressive-like behaviors, and intra-nucleus accumbens administration of HDAC inhibitors (notably the putative class I-selective HDAC inhibitor, MS-275) robustly reverses the stress-induced behavioral phenotype in all three paradigms, similar to the effects observed after chronic treatment with classical antidepressants (e.g., imipramine or fluoxetine). The effect of administration of HDAC inhibitors into the other brain regions is under current investigation, although preliminary analysis indicates partly different effects compared with the nucleus accumbens. To complement these behavioral studies, we are also comparing global patterns of gene expression (via micro arrays) in the nucleus accumbens after MS-275 infusion with that of fluoxetine treatment in previously stressed mice and non-stressed controls. Results from these studies should provide insight into the patterns of gene expression in the nucleus accumbens that may be necessary for relieving depressive behaviors. A direct comparison of manipulations in nucleus accumbens with other limbic structures underscores how these different brain areas contribute unique epigenetic profiles subserving the expression of depressive-like behaviors.

24 Role of DNA methylation in persistent cocaine-induced plasticity in the nucleus accumbens

Quincey LaPlant, Vincent Vialou, Herb Covington, Ian Maze, Ronald Oosting, Geetha Kalahasti, William Renthal, Eric Nestler

Chronic cocaine use induces long-lasting addictive behaviors that can persist for years following drug abstinence. The longevity of these behaviors suggests an underlying neural mechanism of equal stability. We propose that persistent post-translational modification of chromatin and its resultant influence on gene expression functions as a type of cellular memory to ultimately play a crucial role in the maintenance of addictive behavior. In the current study, we focus on DNA methylation, as it is considered to be the most permanent form of chromatin modification. Interestingly, we find that several key DNA methyltransferases and DNA methyl binding proteins are transcriptionally regulated by cocaine in the nucleus accumbens—a key brain reward region. Given such regulation, we next sought out to test the functional relevance that DNA methylation may have on cocaine reward. By utilizing viral overexpression, pharmacological inhibition/activation, and conditional knockout of DNA methyltransferases in the nucleus accumbens, we have assessed the behavioral effect on conditioned place preference (CPP) to cocaine and surprisingly find that DNA methylation functions to inhibit the rewarding properties of cocaine—thereby suggesting that specific genes are methylated and their expression is silenced as a counteractive response to cocaine addiction. Therefore, we next sought to identify gene promoters that are persistently methylated by chronic cocaine. By utilizing cDNA microarrays and chip-on-chip techniques, we report findings of a large number of genes that are indeed hypermethylated and silenced by cocaine. In summary, this work highlights a key role of DNA methylation and gene silencing in suppressing maladaptive cocaine-induced neural plasticity.

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Transcription factor YY1 regulates the myelination of Schwann cellsYe He¹, Jeff Dupree², John Svaren³, Patrizia Casaccia-Bonneli¹¹ Department of Neuroscience, Mount Sinai School of Medicine, New York, NY² Department of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, VA³ Department of Comparative Biosciences, U Wisconsin, Madison, WI

Schwann cell is the myelinating cell in the peripheral nervous system (PNS). Besides its function during development, Schwann cell is capable of promoting neuronal survival and axonal regeneration and remyelination in pathological conditions. These extraordinary properties make Schwann cell a promising candidate for engraft therapy of diseases involves axonal damage and demyelination. Therefore, a better understanding of the molecular mechanism of Schwann cell differentiation will benefit the therapy for both the peripheral neuropathies (i.e. Charcot-Marie-Tooth) and the central nervous system (CNS) diseases (i.e. spinal cord injury).

Previous work in our laboratory has identified the transcription factor Yin Yang 1 as a critical determinant for the differentiation of oligodendrocytes, the myelinating cells of the CNS. To study the function of YY1 in Schwann cell development, *yy1* conditional knockout mice (cko) were generated by deleting *yy1* in Schwann cells using the Cre/loxP system. The *yy1* cko mice displayed paralysis of the hindlimbs starting during the second postnatal week, a time when intensive myelination of the peripheral nervous system occurs. Gross anatomical analysis of the sciatic nerves of *yy1* cko mice revealed defective myelination as revealed by transparent and very thin nerves compared to wild type siblings. Quantitative PCR and immunohistochemistry revealed a dramatic decrease in myelin gene expression. EM studies further confirmed severe hypomyelination in the mutant mice. Detailed analysis of the axonal classification revealed that the mutant Schwann cells established a one to one relationship with the axons (pro-myelinating stage), but rarely proceeded to a more mature stage. This phenotype resembles that observed in Schwann cell master regulator *Egr2/krox20* mutant mice and its binding protein *NAB1/2* null mice. By comparing the gene expression profile of the sciatic nerve of *yy1* cko to *Egr2* and *NAB1/2* mutant mice, we found that these transcription factors may regulate common downstream genes such as *SCIP/Oct-6* and *Id4*. We conclude that YY1 is an essential molecule regulating Schwann cell myelination through *Egr2* pathway.

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**Epigenetic regulation in depression:
studies of chromatin remodeling in mouse nucleus accumbens**

Matthew B. Wilkinson

Though it is a widely studied psychiatric syndrome, major depressive disorder remains a poorly understood illness, especially with regard to the disconnect between treatment initiation and the delayed onset of clinical improvement. We have recently validated chronic social defeat stress in mice as a model in which a depression-like phenotype is reversed by chronic, but not acute, antidepressant administration. Here, we use ChIP-chip assays—chromatin immunoprecipitation (ChIP) followed by genome wide promoter array analyses—to study the effects of chronic defeat stress on chromatin regulation in the mouse nucleus accumbens (NAc), a key brain reward region implicated in depression. Our results demonstrate that chronic defeat stress causes widespread and long-lasting changes in gene regulation, including alterations in repressive histone methylation and in phospho-CREB binding, in the NAc. We then show similarities and differences in this regulation to that observed in another mouse model of depression, prolonged adult social isolation. In the social defeat model, we observed further that most of the stress-induced changes in gene expression are reversed by chronic imipramine treatment, and that resilient mice—those resistant to the deleterious effects of defeat stress—show patterns of chromatin regulation in the NAc that overlap dramatically with those seen with imipramine treatment. These findings provide new insight into the molecular basis of depression-like symptoms and the mechanisms by which antidepressants exert their delayed clinical efficacy. They also raise the novel idea that certain individuals resistant to stress may naturally mount antidepressant-like adaptations in response to chronic stress.

27 Two peptides act via a cAMP/PKA pathway to convert intermediate to ingestive feeding motor programs.

Allyson Friedman

When first stimulated CBI-2 generates an intermediate motor program in which the radula closer motoneuron B8 fires during protraction and retraction, and the radula opener B48 fires at a very low frequency in protraction. With repeated activation of CBI-2 motor programs change. Activity becomes ingestive. B8 fires predominantly in retraction and B48 fires at a high frequency in protraction. Here, we investigate the mechanism underlying the transition from intermediate to ingestive programs.

CBI-2 releases two peptides, FCAP and CP2. In the present study, we found that bath application of these two peptides mimicked the effects of repeated CBI-2 stimulation. Thus exogenous FCAP/CP2 converted intermediate activity to ingestive (increased the B8 firing frequency in retraction and the B48 firing during protraction). Further experiments focused on mechanisms underlying the increased firing of B48. We found that brief stimulation of CBI-2 or the bath application of its two peptides increased the excitability of B48. Importantly, bath application of FCAP and CP2 occluded effects of CBI-2 stimulation, suggesting that excitability increases induced by CBI-2 are mediated by FCAP/CP2.

Other experiments sought to characterize the cellular mechanism of CBI-2 and FCAP/CP2 actions. We found that application of IBMX, a phosphodiesterase inhibitor, extended the duration of the effect of CBI-2 stimulation on B48 excitability. This suggested that the effect of CBI-2 stimulation and FCAP/CP2 could be mediated by cyclic nucleotides. We focused on the effects of the second messenger cAMP. We found that application of forskolin, which raises the level of cAMP increased the excitability of B48. Furthermore, intracellular injection of 8-Br-cAMP mimicked the effects of CBI-2 stimulation and FCAP/CP2 perfusion in that it increased the excitability of B48. In contrast intracellular injections of Rp-cAMP, a PKA inhibitor, blocked the increase of B48 excitability that results from CBI-2 stimulation. We also found that intracellular injection 8-Br-cAMP into B48, converted the low level of B48 activity that is characteristic of intermediate programs into a high level of activity characteristic of ingestive programs. In contrast superfusion of Rp-cAMP blocked the increase of B48 activity in motor programs elicited by repeated CBI-2 stimulation.

Taken together our findings suggest that repeated stimulation of CBI-2 increases the ingestiveness of the pattern of B48 activity by releasing FCAP and CP2 and that in turn the actions of these peptides are mediated via the cAMP/PKA pathway.

28 Double Dissociation and Hierarchical Organization of Strategy Switches and Reversals in the Rat PFC

James J Young, Dr. Matthew Shapiro

Strategy switching and reversal learning are two forms of behavioral flexibility each associated with a different region of the rat PFC – Prelimbic-Infralimbic cortex (PL-IL) and Orbitofrontal cortex (OFC), respectively. To analyze the neural mechanisms of behavioral flexibility in rats, we tested the effects of PL-IL or OFC infusion with the GABA agonist muscimol in the context of multiple strategy switches and reversals. Muscimol infusion into PL-IL impaired retention of strategy switches but not reversals, whereas muscimol infusion into OFC impaired retention of reversals but not switches. Impairment associated with OFC infusion disappeared after repeated reversals, showing that training alters the brain mechanisms of flexible responding. However, the effects of PL-IL and OFC infusion were asymmetric: training in repeated reversals did not improve performance in subsequent strategy switches (Rich and Shapiro, 2007), but training in repeated switches did improve performance in subsequent reversals. These results show that strategy switching and reversal learning mechanisms are not completely independent. The neural systems supporting reversal learning – such as OFC – are engaged during switches even when OFC is not required to perform the task. Learning mechanisms in the rat PFC is organized hierarchically, so that training in flexible responding to generalizes and leads to improved performance across otherwise parallel processes.

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Can fibroblasts be induced to make myelin?Juan Sandoval^{1*}, Jia Liu^{2*}, Gerardo Lopez¹, Sungtao Doh³, Li Cai³ and Patrizia Casaccia-Bonnett²

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The lineage specification and the maturation of oligodendrocytes (OLs), the myelinating cells in the central nervous system, involve the orchestrations of transcription factors (TFs) and post-translational modifications of histones. However, the concept of trans-differentiation from fibroblast to a myelinating cell by a combination of TFs or treatment with epigenetic modifier agents has not been investigated. We have shown that NIH3T3 fibroblasts lack the cell-specific transcriptional activators of myelin genes. Over-expression of a combination of oligodendrocyte-specific TFs, including basic HLH (i.e. Olig1, Olig2, Mash1, E47), homeodomain (i.e. Nkx2.2) and HMG proteins (i.e. Sox10) was able to transiently activate an exogenous myelin basic protein (MBP)-promoter-driven luciferase reporter in NIH3T3 fibroblasts, but not sufficient to induce endogenous myelin gene expression, suggesting the difference in chromatin conformation in myelinating vs. non-myelinating cells. Since the accessibility of chromatin to TFs is dependent on post-translational modification of histones, we sought to define the “histone code” of myelin gene loci in NIH3T3 fibroblasts and compared it with that detected in myelinating cell lines (i.e. Olineu cells) and in primary oligodendrocyte progenitor cultures (OPCs). Chromatin immunoprecipitation (ChIP) analysis in the highly conserved regions of *mbp* and *mag* genes revealed opposite patterns of epigenetic histone modifications related to activation (trimethylation of residue K4 and pan-acetylation of histone H3) and repression (trimethylation of residues K9 and K27 of histone H3), with a prevailed increase in the repressive histone code at the *mbp* and *mag* loci in NIH3T3 fibroblasts. Since methylation of H3K9 and H3K27 is often associated with gene silencing and DNA methylation, we asked whether we could revert the repressive histone code for myelin genes in fibroblasts by treatment with the DNA demethylating agent 5-azadeoxycytidine (5-AzaC) and/or histone deacetylase (HDAC) and sirtuin inhibitors trichostatin A (TSA) and sirtinol. Remarkably, treatment of fibroblasts with 5-AzaC followed by TSA (but not sirtinol) was sufficient to induce the endogenous expression of *mbp*, although at a much lower level than that detected in myelinating cells. This was associated with changes in the histone code at the myelin gene loci and concomitant upregulation of positive (Olig1, Olig2, Sox10 and Mash1) and negative (Hes5 and Id4) regulators of myelin gene expression. These results suggest that global epigenetic modifications are necessary but not sufficient to trans-differentiate a fibroblast into a myelinating cell.

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“STAT3 and SHP2 Integrate Cannabinoid 1 Receptor and Interleukin-6 Receptor Signals to Trigger Neurite Outgrowth”

Yana Zorina, Ravi Iyengar, Kenneth D. Bromberg

Activation of the G_{o/i}-coupled cannabinoid-1 receptor (CB1R) has been shown to induce neurite outgrowth in Neuro2A cells through activation of Src kinase and STAT3 transcription factor. Signaling by the interleukin 6 receptor (IL-6R) also activates STAT3 through Jak kinase. We studied if signals from the two pathways could be integrated in a synergistic manner to trigger neurite outgrowth in Neuro2A cells. We found that CB1R and IL-6R stimulation induced synergistic neurite outgrowth. STAT3 plays a central role in integration of the two signals and is critical for synergistic neurite outgrowth. In addition, signal integration requires the activity of both Jak kinase and Src. When both pathways are activated, STAT3 phosphorylation is sustained for 6 hours. This prolonged activation of STAT3 requires deactivation of SHP2 phosphatase. Reduction of SHP2 levels by RNAi results in greater synergy in neurite outgrowth. Simultaneous knockdown of both SHP2 and STAT3 blocks the synergistic triggering of neurite outgrowth, indicating that STAT3 is downstream of SHP2. Lastly, CB1R and IL-6R co-stimulation enhanced the differentiation of rat cortical neuron primary cultures. These results provide a mechanism of SHP2 – STAT3 interactions in integrating signal from G protein coupled and cytokine receptor to evoke neurite outgrowth in Neuro2A cells.

31**Differentiation of human embryonic stem cells to oligodendrocytes**Kwi Hye Kim¹, Sunita D'Souza², George Diaz³, Yong Zhao³, Patrizia Casaccia^{1,3}¹Department of Neurosciences, Mount Sinai School of Medicine²Department of Gene and Cell Medicine, Mount Sinai School of Medicine³Department of Genetics and Genomics, Mount Sinai School of Medicine

Recent advances in the methods of reprogramming of somatic cells into stem cell-like states have opened gates to the possibilities on the study of genetic disorders of ill-defined pathogenesis and the development of personalized therapies. In this study, we are aiming to establish a protocol to differentiate human embryonic stem cells (hESC) and patient-specific induced pluripotent stem cells (iPSC), into oligodendrocyte progenitor cells to study the pathogenesis of white matter disorders in cobalamin C deficiency. To establish a standardized protocol of differentiation, human embryonic stem cells H1 have been differentiated employing several methods that have been previously published and widely accepted. When the most popular neural differentiation method of hESC starting with ESC sphere, which is also termed as embryoid body formation, we could achieve rosette and neural tube-like structure, a morphological signature of neural differentiation of hESC. These structures were characterized by a population of Pax6 positive neural lineage cells. The most promising results were, however, obtained when the hESC on the Matrigel®-coated surface were differentiated in the presence of retinoic acid, noggin and sonic hedgehog. At the end of the 6-day treatment with factors mentioned above, ES cells were both nestin and Pax6 positive, thereby indicating the presence of neural stem cells. One of the obstacles, however, remains the heterogeneity of the cell population at any time point. To overcome this obstacle and obtain pure cell populations for each development stage, we are working on perfecting the bacterial artificial chromosome (BAC) technology and generate reporter lines, expressing the appropriate stage-specific markers. The long term goal is to generate multiple lines of oligodendrocytes from patients with distinct genetic mutations in the *cb1c* gene.

32 **Opposing roles of BMP4 and SHH in modulating oligodendrocyte progenitor cell fate.**Ju Wang¹, Siming Shen², Tung Nguyen³, Ioannis Androulakis⁴, Patrizia Casaccia^{1,*}¹ Department of Neuroscience, Mount Sinai School of Medicine, One Gustave Levy Place, box 1065, New York, NY 10029² Department of Neuroscience and Cell Biology, RWJMS, Piscataway, NJ 08854³ BioMaPS Institute for Quantitative Biology, Rutgers University, Piscataway, NJ 08854⁴ Biomedical Engineering Department, Rutgers University, Piscataway, NJ 08854* Correspondence: Patrizia.casaccia@mssm.edu

We have previously shown that histone deacetylation, mediated by the activity of histone deacetylases is necessary for the differentiation of oligodendrocyte progenitor cells (OPC). In this study we asked whether stimuli that are known to favor oligodendroglialogenesis (Shh) or antagonize it (BMPs) also modulate HDAC activity. Shh positively regulates the differentiation of A2B5+ OPCs into O4+ oligodendrocytes (OLs) and increases HDAC activity at the 24 hour time point, while BMP4, that antagonizes OPC maturation and promotes the lineage progression towards astroglialogenesis, decreases it. Interestingly, the time course of HDAC activity in Shh treated cells displays an early decrease followed by the subsequent increase. Pharmacological inhibition of HDAC activity or silencing of specific isoforms prevents Shh-mediated oligodendroglialogenesis while facilitating BMP4-mediated astroglialogenesis. To define the downstream genes responsible for these effects, we conducted gene profiling studies using Affymetrix microarray of genes differentially affected by HDAC activity in Shh and BMP treated OPCs over time. We therefore applied t-test statistics to filter which genes are significantly up- or down- regulated and then combined with our prior knowledge and the literature e.g. gene ontology and pathway enrichment to identify potential target genes for further analyses. Several genes were identified, and now we are validating these candidate targets in cultured progenitors.

33 Impaired autophagy in dopaminergic neurons results in progressive axonal degeneration associated with Parkinson's disease

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Parkinson's disease (PD) is characterized by the formation of cytoplasmic inclusion bodies (Lewy bodies) containing ubiquitinated proteins, including alpha-synuclein. The toxic accumulation of alpha-synuclein contributes to the appearance of dystrophic axon terminals and the loss of dopaminergic (DA) neurons in the midbrain. Intact intracellular catabolism is therefore crucial for regulating protein levels of these cells. Autophagy, the lysosomal degradation of protein complexes/aggregates and cellular organelles, is one such clearance mechanism. Growing evidence reveals that autophagy is constitutively active in the central nervous system. We have recently shown that genetic ablation of autophagy in Purkinje cells leads to cell-autonomous axonal dystrophy and degeneration. To examine whether the neuroprotective role of autophagy extends to the maintenance of dopamine homeostasis and protein turnover in catecholaminergic axon terminals, we established conditional knockout mice containing the cell-specific deletion of Atg7, an essential autophagy gene, in neurons expressing tyrosine hydroxylase (TH). Inactivation of Atg7 enhanced protein levels of alpha-synuclein in whole-brain lysates and augmented the formation of ubiquitin aggregates in the substantia nigra. TH and SMI-31 immunostaining of the dorsal striatum indicated the presence of dystrophic axons in autophagy-deficient mice as early as one month old. Nigrostriatal axons terminating on medium spiny neurons progressively degenerated through 10 months of age. Furthermore, TH-positive cell loss in the substantia nigra of knockout mice was not observed until after one year. Our results suggest that autophagy is critical in maintaining alpha-synuclein turnover in nigrostriatal neurons. When autophagy is dysfunctional, these dopaminergic neurons undergo a progressive axonal degeneration that precedes midbrain cell body death.

34 Early postnatal deletion of N-cadherin alters molecular composition and plasticity of adult hippocampal synapses

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It is well documented that the synaptically-enriched cell adhesion molecule N-cadherin has an obligatory role in early brain development and establishment of synaptic connectivity. However, its role in maintaining synaptic form and function beyond initial synaptogenesis and in maturity is unknown. To address this, we used a loxP-Cre recombinase strategy to generate mice harboring a conditional (postnatal-, forebrain- and excitatory neuron-specific) deletion of N-cadherin which commences in the third postnatal week, bypassing confounding antecedent roles of N-cadherin in development of basic brain structure, migration, axon growth and synaptogenesis. Immunocytochemistry was used to confirm the removal of N-cadherin from hippocampal regions CA1, CA3 and dentate gyrus (DG) of these conditional knockout mice. Quantitative analysis of immunolabeling for various pre- and postsynaptic molecular markers in hippocampus from adult (4-6 month old) N-cadherin knockout mice showed significantly decreased numbers of synaptic puncta in all regions (CA1, CA3, and DG) compared to Cre controls. Those that remained were smaller in area when compared to those in Cre controls. Such markers included postsynaptic density protein-95 (PSD-95), AMPA-type glutamate receptor subunits (GluR) 2/3, and vesicular glutamate transporters (vGluts) 1, 2, and 3. Functionally, early postnatal ablation of N-cadherin impaired stability of long-term potentiation (LTP) and LTP-associated dendritic spine enlargement following theta burst stimulation when examined in adulthood, but several forms of long-term depression (LTD) were unaffected. Together these data suggest that N-cadherin has a continuous role, beyond initial development, in maintaining synapse number and/or molecular composition, deficits of which lead to impaired synaptic structural and functional plasticity in adulthood of the kind critical for higher cognitive function.

35 A Biochemical Correlate for the Inverted-U Effect of Stress on Memory Consolidation

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The process by which newly learned information becomes a long lasting memory is termed memory consolidation. This process requires the activation of the CREB-C/EBP (cAMP Response Element Binding protein – CCAAT/Enhancer Binding protein) cascade as well as the function of a number of plasticity related proteins such as phosphorylated calcium-calmodulin kinase II (pCamKII), activity-regulated cytoskeletal protein (ARC), brain-derived neurotrophic factor (BDNF) and glucocorticoid receptors (GRs) in the hippocampus. Traumatic experiences are known to alter the mechanisms of memory stabilization and strengthening leading to persistent and intrusive memories of the traumatic events. A large body of literature indicates that stress and glucocorticoid hormones modulate memory strength in an inverted-U dose response manner: optimal enhancing effects are seen at moderate doses while high doses impair memory retention. The molecular mechanisms underlying this inverted-U effect of stress still remain to be determined. In this study, we seek to delineate the relationship between the inverted-U curve effect of stress on memory formation and the activation of molecular mechanisms known to underlie memory consolidation, including CREB, C/EBP and plasticity related proteins. Animals were trained in the inhibitory avoidance paradigm with various shock intensities to establish IA protocols that evoke memory retention that follows the inverted U profile. We then determined whether the strength of the memory correlated with the expression levels of pCREB, pCamKII, ARC, BDNF and GR in the hippocampus during memory consolidation. Quantitative western blots from dorsal hippocampal extracts revealed a correlation between memory retention and the expression levels of the various plasticity markers. Thus, the memory decline seen with the high intensity shock training is accompanied by a decrease in the levels of the markers tested. These profiles were seen at both 1hr and 20hrs after training indicating that the changes in CREB, C/EBP and plasticity related proteins follow an inverted-U curve throughout the phase of memory consolidation. Our data suggest that traumatic amnesia reflects a lack of hippocampal memory consolidation.

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36 A mutant $\delta 2$ ionotropic glutamate receptor exhibits dual regulation by phosphoinositidesVasileios I. Petrou^{1,2} and Diomedes E. Logothetis².

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The $\delta 2$ glutamate receptor (GluR $\delta 2$) is considered a member of the ionotropic glutamate receptor family, although a specific ligand that activates the wild-type receptor has yet to be identified. GluR $\delta 2$ is enriched in the parallel fiber-Purkinje cell (PF-PC) synapse, but the precise physiological role of the receptor is still unclear. A naturally-occurring single point mutant in the third transmembrane domain of the receptor (A654T), named Lurcher (GluR $\delta 2^{Lc}$), exhibits constitutive activity.

Our previous preliminary results suggested that the $\delta 2$ glutamate receptor - Lurcher mutant is inhibited by direct interactions with phosphatidylinositol 4,5-bisphosphate (PIP₂). Here we show that pre-incubation with wortmannin affects the activity of the receptor in a concentration-dependent manner, leading to an inhibition of the channel in the low μ M range. This suggests that PI3K could also be involved in the regulation of the $\delta 2$ glutamate receptor. We further investigate the involvement of phosphoinositides in the regulation of $\delta 2$ glutamate receptor using more specific PI3K and PI4K inhibitors.

37 **Δ FosB and synaptic plasticity in the NAc mediates resiliency to stress**

Vialou V, LaPlant QC, Covington H, Dietz D, Ohnishi Y, Nestler EJ

Why some people succeed in coping with adverse situations that are detrimental to human psychological development while others are more vulnerable to stressful situations? Due to the lack of good animal models, it has been studied mostly through the point of view of psychology. Studies done in specific population like patients suffering from PTSD have underlie some biological markers of susceptibility or resistance to stress. But the extent to which these factors are a cause or a consequence of susceptibility/resilience remains undetermined. The identification of the molecular mechanisms of susceptibility or resiliency might help us treat stress-induced mood-related disorder like anxiety or depression.

The transcription factor Δ FosB play an important role in the nucleus accumbens in mediating the motivation toward natural stimuli and drugs of abuse. Here, we found by use of immunohistochemistry that Δ FosB is induced in the nucleus accumbens by chronic social defeat particularly in resilient mice. Several lines of evidences support the idea that this induction of Δ FosB is protective by helping the individual cope with stress. Inducibly overexpressing Δ FosB in the adult nucleus accumbens and dorsal striatum in bitransgenic mice, and specifically in nucleus accumbens by use of viral-mediated gene transfer, reduced the aversive effect of social defeat. Social isolation induces a reduction in Δ FosB levels in the nucleus accumbens and promotes vulnerability toward social defeat. This avoidance-induced by acute defeat in isolated mice can be reversed by the overexpression of Δ FosB, suggesting a therapeutic effect. Indeed, chronic administration of fluoxetine, a standard antidepressant, also induces Δ FosB in the nucleus accumbens. Finally, we found by western blot that depressed humans have diminished Δ FosB levels in the nucleus accumbens.

Δ FosB play an important role in brain plasticity by inducing or repressing a variety of genes. One of these targets is the AMPA receptor subunit GluR2. Several line of evidence support the idea that decrease in NAc firing may be related to reward. Here, we demonstrated that general dampening of NAc neurons activity via microinjection of NBQX (an AMPAR antagonist) or via overexpression of GluR2 reduces the social-defeat induced avoidance. This correlates with the opposite regulation in GluR2 levels observed in defeated and resilient mice.

In conclusion, our data demonstrates the essential role of Δ FosB in mediating the synaptic plasticity necessary for the coping response to chronic stress.

38**Imaging MSBs In Vitro Using Confocal Microscopy and Retrospective EM**

James Reilly, Phillips Lab

In embryonic and neonatal hippocampi, most glutamatergic excitatory synapses are on dendritic shafts but in adult hippocampi, most are on dendritic spines. How the shift from mostly shaft to mostly spinous synapses occurs is not known. For this shift to occur, we hypothesize that spinous synapses proliferate by multisynaptic bouton (MSB) resolution into new spinous synapses. A multisynaptic bouton (MSB) is a presynaptic bouton with multiple postsynaptic contacts. MSBs have only so far been described using electron microscopy (EM). To test our MSB resolution hypothesis, we are using laser-scanning confocal microscopy (LSCM) to live-image MSBs in hippocampal neuron cultures. This method provides feasibility for several techniques: transfecting multiple fluorescent markers, simultaneously imaging many bouton-spine contacts over time (hours to weeks) at high resolution, and relocating dendritic segments for retrospective EM or additional live-imaging. In our cultured neurons, about 6-8% of synapses are likely to be MSBs as identified by LSCM of immunolabeled synaptic profiles. Because MSBs have only been described using EM, we are doing serial section retrospective EM of LSCM-imaged spines to establish criteria for identifying MSBs by LSCM. To make the criteria as accurate as possible, synapses in both the EM serial sections and LSCM z-stacks are reconstructed in 3D. We are using LSCM to live-image MSBs in presynaptically marked neurons co-cultured with postsynaptically marked ones over several hourly, daily, and weekly time courses.

39 **The E2F family of transcription factors mediate the transcriptional regulation necessary for oligodendrocyte differentiation**

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Oligodendrocytes are responsible for forming myelin sheaths in the central nervous system. The full expression of myelin genes does not occur until after cell cycle exit, therefore an obligatory relationship exists between cell cycle exit and oligodendrocyte differentiation. The mechanism of how these two events are coupled is unknown. We hypothesize that transcriptional co-regulation of cell cycle molecules and chromatin regulators could couple these two events. This study investigates the possible roles of E2F transcription factors in mediating this transcriptional regulation. In silico analysis reveals an enriched capability of E2F binding to the promoters of co-expressed genes involved in cell cycle and chromatin regulation. ChIP analysis confirms that E2F family members are recruited to the promoters of many of these genes and reveals a switch in which E2F family member is bound upon differentiation. E2F1 is bound to active promoters in proliferating OPCs while E2F4 replaces E2F1 in differentiating cells. These data suggest that the switch in the predominant E2F family member from E2F1 to E2F4 might be necessary for oligodendrocyte differentiation. This hypothesis is confirmed by silencing E2F4 with siRNA. Furthermore we have found that a gene downstream of E2F regulation, Uhrf1, is able to directly regulate the expression of the myelin gene Mog. This study provides a mechanistic link between cell cycle exit and the initiation of a genetic program of differentiation within the central nervous system.

40 **Genetic basis of lifespan and obesity: Role CtBP2, CBP, CREB1, and sensitivity to glucose**

Michael M Poplawski and Charles V Mobbs.

Twin studies have demonstrated that propensity for short lifespan and obesity are both heritable, but genome wide scanning studies have clearly demonstrated that polymorphisms in no single gene could account for more than a very small fraction of the variance in each trait. However, several lines of evidence suggest that both lifespan and propensity for obesity are largely determined by neuroendocrine, especially hypothalamic, mechanisms. To search for genes that might mediate genetic effects on lifespan and obesity, we screened hypothalamic expression of genes implicated in aging and energy balance for correlation with lifespan and weight gain on a high-fat diet across 5 inbred strains of mice. To date, we have measured mRNA expression levels in the hypothalamus of five mouse strains for over 30 genes implicated in aging and energy balance. Only two genes have so far exhibited a significant positive correlation with lifespan: CREB1 and CBP; interestingly, expression of these genes also correlates positively with weight gained on a high-fat diet. Only one gene correlates negatively with lifespan: CtBP2; interestingly, expression of this gene correlates negatively with weight gain on a high-fat diet. Several lines of evidence suggest that these genes may interact reciprocally to regulate hypothalamic sensitivity to glucose. We have gone on to demonstrate that our standard mouse strain, C57Bl/6J is highly sensitive to hypoglycemia, thus relatively insensitive to glucose, and is also the longest-lived strain and most sensitive to diet-induced obesity. Conversely, the shortest-lived strain, A/J, is almost completely insensitive to hypoglycemia, thus presumably is highly sensitive to glucose, and is also the shortest-lived and least sensitive to diet-induced obesity. We hypothesize that a difference in hypothalamic glucose sensitivity, mediated by differential CtBP2 expression, could account for genetic effects on lifespan and diet-induced obesity.

41

FosB is essential for the enhancement of stress tolerance by Δ FosB

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fosB gene is transcribed into two mature mRNA via alternative splicing, and they encode FosB and Δ FosB/ Δ 2 Δ FosB proteins, respectively. Expression of FosB is transient, while Δ FosB/ Δ 2 Δ FosB chronically accumulates in specific brain regions with prolonged stress or repeated stimuli like drug of abuse. To understand the biological significance of FosB and Δ FosB/ Δ 2 Δ FosB independently, we established fosB^{d/d} (d/d) mice which express only Δ FosB/ Δ 2 Δ FosB from endogenous fosB gene, and fosB-null (G/G) mice. Beyond expectation, we detected intensive accumulation of Δ FosB/ Δ 2 Δ FosB response to spontaneous stimuli in fosB^{d/d} and fosB^{+/d} (+/d) mice, indicating that +/d mice are model for endogenous Δ FosB accumulating mice, and that the difference between d/d and +/d mice discloses the role of endogenous FosB under Δ FosB/ Δ 2 Δ FosB accumulation. From behavioral examinations with these mice, G/G mice exhibit hypoactivity, and higher stress vulnerability, suggesting that fosB gene products exert an antidepressant-like effect. +/d mice exhibit hyperactivity and higher stress tolerance, suggesting that accumulated Δ FosB/ Δ 2 Δ FosB induces an antidepressant-like state. d/d mice exhibit extremely hyperactivity and higher dopamine sensitivity, but stress vulnerability similar to that seen in wild-type mice, indicating that their behavioral pattern is similar to bipolar disorders, and that FosB is essential for stress tolerance by Δ FosB/ Δ 2 Δ FosB accumulation.

Additionally, we have established a new mouse line, in which the 3' UTR of the fosB gene has been truncated. 3' UTRs can be very important for both mRNA stability and translation. For this reason, in these mutant mice (termed fosB^{nA/nA} (nA/nA) for non-accumulating), the fosB gene expresses no FosB, normal baseline levels of Δ FosB but no further induction of this variant, and higher levels of Δ 2 Δ FosB. The main difference in fosB gene expression between d/d and nA/nA is whether Δ FosB is able to accumulate or not, suggesting that the molecular and behavioral differences between d/d and nA/nA mice will disclose the functional significance of Δ FosB, not Δ 2 Δ FosB. nA/nA mice show lower activity than G/G mice, and similar hypotolerance against repeated forced swim test to that in G/G mice, suggesting that accumulated Δ 2 Δ FosB can not complement the effect of accumulated Δ FosB in locomotion activity and stress tolerance.

These data suggest fosB gene has a potential to regulate mood disorders-related behavior.

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Modulation of Intracellular Calcium Concentration During Motor Activity in Aplysia Mechanoafferent B21

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Signal transmission from the mechanoafferent B21 to its follower motor neuron B8 is regulated via phasic changes in membrane potential during feeding motor programs. During the protraction phase of such programs, B21 is either hyperpolarized or is at its resting membrane potential and afferent transmission does not occur. During the retraction phase of the motor program, B21 is depolarized and afferent input is gated in, i.e. afferent transmission to B8 occurs. A goal of our research is to characterize the mechanisms underlying this phase dependent afferent control.

Previously we have shown phasic depolarization that is subthreshold for spike initiation and can induce an increase in the intracellular calcium concentration in readily imaged parts of B21 such as its two primary processes. This calcium increase could promote afferent transmission by potentiating B21-B8 synaptic transmission if it also occurs in the region which is the presumed site of contact with B8, the secondary and tertiary branches of B21's lateral process. To determine whether this is the case, we used calcium-imaging to measure calcium concentration changes induced by central depolarization. With 40x imaging we were able to detect calcium increase throughout higher order branches of the lateral process.

To determine whether this increase will be observed under physiological conditions, we characterized phasic depolarization in B21 during motor programs in the isolated nervous system. The depolarization was approximately 15 mV in the lateral process and soma of the cell. We then mimicked this depolarization, using a correction factor that took into account the lateral process length constant. A significant increase in calcium concentration was detected in the lateral process.

In final experiments we compared the magnitude of the change in calcium concentration induced by subthreshold depolarization, to the magnitude of the change in calcium concentration induced by a type of afferent activation of B21. In this case afferent activation consisted of single spikes triggered by peripherally applied mechanical stimuli. We found that the calcium increase induced by individual spikes was similar in magnitude to the increase caused by the central subthreshold depolarization.

In conclusion our data suggest that motor program induced subthreshold alterations in membrane potential are likely to produce a significant increase in intracellular calcium concentration in the region of B21 that makes contact with the follower B8. This phasic increase is likely to potentiate synaptic transmission and to contribute to the phasic control of afferent transmission that occurs during feeding motor programs.

43 “Regulation of human APP metabolism in neuroblastoma cells by novel signal transduction pathways.”

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Biochemical pathways regulating the metabolism of the amyloid precursor protein (APP) and the amyloid β (A β) peptide have become the primary targets for drug development in Alzheimer’s Disease (AD) over the past two decades. Work from our lab provides compelling evidence for competitive regulation of APP metabolism by amyloidogenic versus non-amyloidogenic pathways. Processing of APP results in a secreted ectodomain (sAPP α or sAPP β) and membrane bound C-terminal fragment (CTF) following cleavage by α - or β -secretase. The α -cleaved CTF is processed by γ -secretase to produce a non-amyloidogenic p3 peptide, whereas the β -cleaved CTF is processed by γ -secretase to produce the amyloidogenic 39-43 amino acid A β peptide. Using stably transfected neuroblastoma N2a cell lines expressing either wild-type or Swedish mutated APP, we evaluated regulation of APP metabolism by novel signal transduction pathways. It is well established that activation of protein kinase C (PKC) occurs downstream of many signaling pathways, including receptor tyrosine kinases and many neurotransmitter receptors. Activation of PKC by phorbol-12,13-dibutyrate (PDBu) increases processing of APP via the non-amyloidogenic pathway, decreasing amyloidogenic products. Doody et al (2008) recently published promising results from a phase II randomized clinical trial of the drug dimebolin (Dimebon®) – a compound which acts on a variety of pharmacological targets including histamine and monoamine receptors, L-type calcium channels, mitochondrial pores, and acetylcholine esterase. Dimebolin’s compelling clinical profile and evidence that many of these pharmacological targets are known modulators of APP and A β metabolism led us to evaluate whether dimebolin modulates APP metabolism in cultured cell lines. We are the first to show that dimebolin indeed regulates APP metabolism in both acute and chronic treatments, and long-term treatment with dimebolin (in cultured cells) activates the non-amyloidogenic pathway. Finally, recent evidence suggests that Rho family GTPases (Rho, Rac, CDC42) modulate maturation and metabolism of APP and we present preliminary evidence that CDC42 may modulate trafficking and processing of holoAPP. Taken together, we present several new target pathways for modulation of APP metabolism in the treatment of AD. Future work will elucidate the effects of dimebolin on A β clearance and toxicity, as well as regulation of metabolism within a more aggressive model of AD.

2009 UPCOMING EVENTS

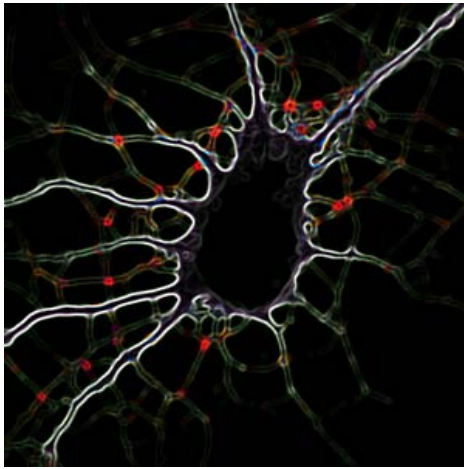


photo by Greg Phillips

<i>July</i>	<i>August</i> Graduate School Orientation
<i>September</i>	<i>October</i> Society for Neuroscience Meeting
<i>November</i>	<i>December</i>

Graduate Program information

This has been a benchmark year for the Neuroscience MTA as it continues to grow in strength, flexibility, and stature. Notably, there have been several important changes implemented or planned to accommodate the ever-increasing diversity, talents and interests of our students. First, starting this fall ('09), the Neuroscience Core classes will be taught at 9:00 am. This change came about partly in response to student's wishes (those questionnaires do count!), and partly to accommodate the Neuroscience-MSTP students, who will now be able to take the Neuroscience Core class sequence during their first medical school year in conformity with other MTA schedules. Second, new Neuroscience Core class co-Directors will provide fresh energy and leadership for the Core class sequence starting this fall ('09). Systems Neuroscience (Core 1) will be co-Directed by Betsy Cropper and Patrick Hof; Cellular and Molecular Neuroscience (Core 2) will be co-Directed by Cristina Alberini, Greg Phillips, and Marianna Max, and the Neural Basis of Behavior (Core 3) will be co-Directed by Matthew Shapiro and Mark Baxter, one of Neuroscience's

newest recruits. In addition, Neuroscience Journal Club, our critical 'work in progress' forum that is required for all Neuroscience students, will now be co-Directed by Scott Russo and Jenny Zou, a new Neuroscience/Neurosurgery recruit. Third, look for a number of new advanced Elective courses that will complement currently offered Electives. While final scheduling details are now being worked out, students will have a broad repertoire to choose from starting this fall ('09), including Neuropharmacology (Nestler and Hurd); Brain Imaging: In vivo methods (Kaplan); Advanced Topics in Synapses (Benson); Advanced Topics in Cognition (Shapiro and Baxter); Neurodegeneration (Yue); Biophysics of Membranes and Membrane Proteins (Max); Cellular Physiology and Ion Channels (Brezina); Neuroanatomy (Holstein); Neurobiology of Aging and Adult Development (Mobbs); Advanced Signal Transduction (Hirsch and Iyengar); The Science Rave (a new hands-on methods course; Cagan and Soriano); and others. Many of these courses will be/are offered every other year, so students should keep their eyes and ears open and plan ahead, so they don't miss the opportunity to take a

particular advanced elective of interest. Fourth, the Qualifying Examination has been streamlined, and will consist only of a comprehensive and integrative oral exam on material covered in the Neuroscience Core class sequence. Finally, under the Directorship of Steve Salton and Eric Nestler, a Neuroscience Training Grant supporting graduate students in their first two years of study has been awarded by the National Institute of Mental Health. This is the program's first-ever Training Grant, and represents a significant achievement.

George Huntley
and Stephen Salton

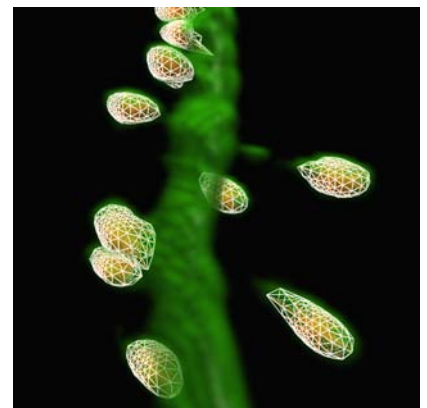


photo by the Wearne laboratory