

THE

2010

Friedman Brain Institute

and the NEUROSCIENCE TRAINING AREA

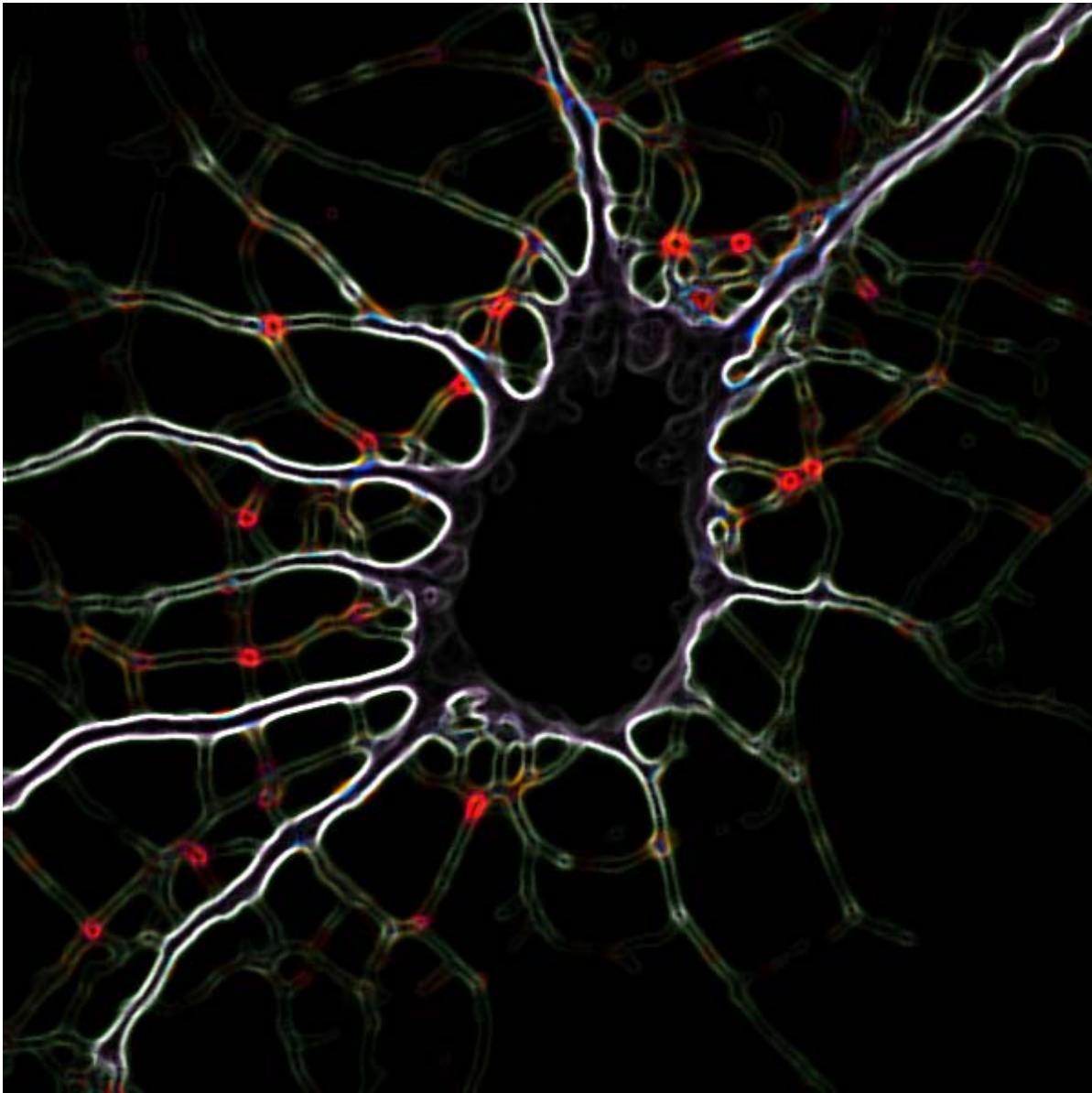
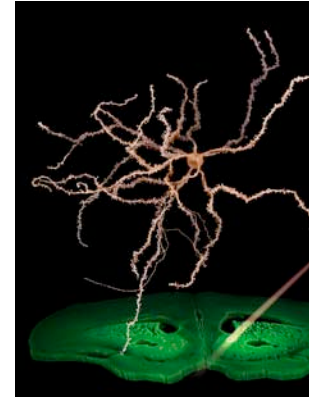
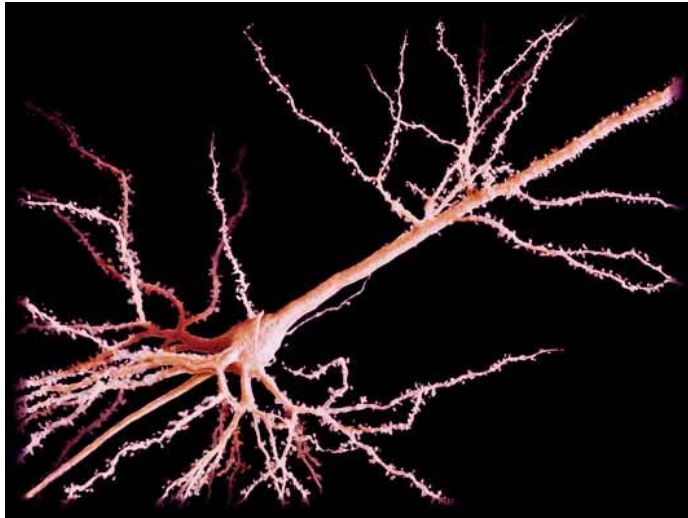


photo by Greg Phillips

Mount
Sinai

SECOND ANNUAL NEUROSCIENCE RETREAT



*photo by the Dani Dumitriu
and TheVisualMD*

photo by John H. Morrison

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Neuroscience Retreat Schedule

NEW YORK ACADEMY OF MEDICINE 1216 Fifth Avenue (corner of 103rd Street)

9:30am..... Continental Breakfast: Entry Hall and Room 20A/B, 2nd fl.
Poster setup: Library 3rd fl.

Hosack Hall

10:00am.....Eric Nestler

10:35am.....Stephen Salton / George Huntley

10:45am.....Keynote Address: Ron Alterman
“Functional Neurosurgery: State of the Art”

SESSION 1- CHAIRPatrizia Casaccia, Hosack Hall

11:45am..... Amir S. Bahar (Neuroscience)

12:00pm..... Xiaosi Gu (Psychiatry and Neuroscience)

12:15pm..... Valentina Dilda (Neurology)

12:30pm..... Christoph Buettner (Medicine and Neuroscience)

LUNCH 12:35pm - 1:40pm, Room 20A/B, 2nd fl.

SESSION 2- CHAIRPatrick Hof, Hosack Hall

1:40pm.....Patrick Hof

1:50pm.....Lauren Friedman (Neurology)

2:05pm.....AJ Robison (Neuroscience)

2:20pm.....Yana Zorina (Pharmacology)

2:35pm.....Jose L Moreno (Pharmacology)

2:50pm.....Miguel A. Sosa (Psychiatry)

POSTER SESSION Library 3rd fl.

3:15pm.....Poster Session Begins

4:00pm.....Posters and Reception

5:30pm.....Best Poster Award: Selected by a jury of peers.

Each attendee is asked to vote for what he/she deems is the best poster.

6:00pm.....Reception Ends

Abstracts

1 **Dynamic CA1 and CA3 coding reflects generalization and discrimination across new and established episodic memories**

Amir S. Bahar and Matthew L. Shapiro

Department of Neuroscience, Mount Sinai School of Medicine, New York, NY, USA

Our ability to generalize between different experiences (e.g., I know it's my office even if my chair is missing from it), or to discriminate between experiences (e.g., many rooms have a table and a chair, but I know they are not my office) is essential in our daily life. How does the brain perform these conflicting cognitive operations? We found evidence that neural activity in CA1 and CA3 hippocampal layers differentially encoded generalization and discrimination between new and established episodic memories. Specifically, activity of CA3 cells reflected generalization while neural activity in populations of CA1 cells reflected an active discrimination process that was correlated with correct memory performance. Thus, hippocampal neural activity reflected the convergence of multiple cognitive operations even if they are in conflict.

2 **Novel aspects of brain insulin: A tale of fat and inflammation.**

Christoph Buettner MD, PhD, Departments of Medicine and Neuroscience.

The CNS plays an important role in the regulation of energy homeostasis. Central pathways also orchestrate innate immunity via the autonomic nervous system, in part through the cholinergic anti-inflammatory reflex. Our interest is to understand how hormones and nutrients are sensed by the hypothalamus and how the brain regulates nutrient partitioning in peripheral tissues like liver and adipose tissue and also how these same pathways impact the inflammatory state of an organism. In rodent models of obesity and in type 2 diabetes this brain control of metabolism is impaired and we believe this dysfunction plays an important role in the pathogenesis of both of these conditions. Interestingly, diabetes and obesity are also associated with a pro-inflammatory state.

We have combined stereotactic infusions with euglycemic clamps and metabolic tracer dilution techniques to assess whole body lipid and glucose fluxes. We find that insulin, through signaling in the mediobasal hypothalamus regulates lipolysis in adipose tissue by regulating sympathetic nervous system outflow. Endocannabinoids can act as retrograde inhibitors within the CNS and are known to be elevated in obesity and diabetes in the hypothalamus. Here we show that icv infused endocannabinoids impair the ability of systemic insulin to suppress hepatic glucose production and to suppress lipolysis. Thus, endocannabinoids are sufficient to impair brain insulin action with detrimental metabolic consequences such as unrestrained lipolysis. A second novel aspect of brain insulin signaling is that it regulates innate immunity and exerts systemic anti-inflammatory effects. Icv insulin improves survival in a LPS model of sepsis in mice which may in part explain the beneficial effects of intensive insulin treatment in critically ill patients. Thus, brain insulin resistance may be the basis for the link between impaired metabolic control and inflammation in diabetes and obesity.

3 Tolerance and Cognitive Performance during Galvanic Vestibular Stimulation (GVS)

Valentina Dilda¹, Bandar Hakim¹, Hamish G. MacDougall² and Steven T. Moore¹

¹Department of Neurology, Mount Sinai School of Medicine, New York, NY, USA;

²Department of Psychology, University of Sydney, Australia.

We have validated Galvanic vestibular stimulation (GVS) as a ground-based analog of microgravity-related sensorimotor deficits, such as postural and gait instability, and impairment of pilot performance during space shuttle landings. The stimulus consists of a small (5 mA peak) pseudorandom electrical current delivered across the mastoid processes with surface electrodes. The aims of the present study were to quantify tolerance to GVS and assess the effects of Galvanic stimulation on cognitive performance. Subjects received one of two peak current levels; 3.5 mA (N=30) and 5 mA (N=30), over a period of 12 min. Subjects performed a battery of cognitive tests before, during, and after GVS exposure. The cognitive battery included reaction time, dual tasking, mental rotation, matching to sample, perspective taking, Stroop, and manual tracking. Motion sickness symptoms were monitored throughout the experiment. GVS was well tolerated; 92% of subjects (55/60) experienced no significant adverse effects to 12 minutes of GVS. Consistent with prior in-flight studies on the effects of microgravity on cognitive function, there was no effect of GVS on reaction time, dual tasking, mental rotation, Stroop, and manual tracking. There was a significant increase in errors during the match to sample task (requiring subjects to match a previously viewed 2D shape with two closely matching samples) and during perspective taking (where subjects were required to indicate a compass direction relative to the orientation of an aircraft icon on a map). These results indicate that acute GVS exposure interferes with short-term memory and complex spatial tasks. GVS is well tolerated, and, like microgravity exposure, does not affect basic cognitive performance. However, performance on spatially demanding tasks, which have been shown to be impaired in-flight (perspective taking was implicated in the Progress collision with the Mir space station), were affected by GVS. Funded by NSBRI through NASA NCC 9-58

4 Inactivation of Autophagy Causes Progressive Degeneration of Dopamine Neurons and Impaired Clearance of Alpha-Synuclein in Mice

Lauren G. Friedman¹, Lenard M. Lachenmayer¹, Liqiang He¹, Shibu Poullose¹, Gay R. Holstein¹, and Zhenyu Yue¹

¹Departments of Neurology and Neuroscience, Mount Sinai School of Medicine, New York, New York, USA

Parkinson's disease (PD) pathology is characterized by the formation of cytoplasmic inclusion bodies (Lewy bodies) containing ubiquitinated proteins, such as alpha-synuclein, 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 and is neuroprotective. Previously we showed that the genetic ablation of autophagy in Purkinje cells leads to cell-autonomous axonal dystrophy and degeneration. To investigate the neuroprotective role of autophagy in dopaminergic (DA) neurons and the pathogenesis of PD, we established conditional knockout mice containing the cell-specific deletion of Atg7, an essential autophagy gene, in neurons expressing tyrosine hydroxylase. Inactivation of Atg7 resulted in enhanced protein levels of alpha-synuclein in whole-brain lysates and augmented the formation of ubiquitin aggregates in both somata and dendrites of the substantia nigra. While autophagy-deficient mice exhibited early axonal and dendritic dystrophy and reduced striatal dopamine content, locomotor deficits and the loss of midbrain DA neurons were observed at later time points, suggesting the slow progressive degeneration of midbrain neurons. These results suggest that autophagy is a critical catabolic mechanism that prevents toxic oligomeric alpha-synuclein formation and degeneration of axons/dendrites of midbrain dopamine neurons. Our study not only supports the recent evidence that impaired autophagy (especially mitophagy) contributes to some familial form of PD, but also implicates dysfunctional autophagy in the pathogenesis of sporadic PD.

NIH/NINDS R01NS060123-01A1 and The Michael J. Fox Foundation for Parkinson's Research

5 DIFFERENTIAL INTERACTIONS BETWEEN NEURAL PROGENITORS AND ENDOTHELIAL CELLS IN THE DEVELOPMENT OF THE BRAIN.

Miguel A. Gama Sosa^{1,4}, Rita De Gasperi^{1,4}, William Janssen³, Patrick R. Hof³ and Gregory A. Elder^{1,2,5}. Departments of Psychiatry¹, Neurology² and Neuroscience³, Mount Sinai School of Medicine of New York University, New York, NY, 10029; Research & Development⁴ and Neurology⁵ Services, James J. Peters Department of Veterans Affairs Medical Center, Bronx, NY 10468.

To study neural/endothelial cell interactions we have developed a procedure to simultaneously isolate neural progenitor and endothelial cell fractions from embryonic mouse brains. Depending on the culture conditions endothelial cells were found to favor maintenance of the undifferentiated neuroprogenitor phenotype through the production of soluble factors, or to promote neuronal differentiation through direct contact in a process mediated by the extracellular matrix protein collagen IV *via* FAK/ERK signaling. In addition, we found cilia in E15.5d brain endothelial cells *in vivo* and *in vitro*. Some of these cilia were motile. Direct contact of endothelial cells with neural progenitors resulted in the loss of endothelial cilia. These results illustrate the differential cellular interactions necessary for the normal development of the brain.

6 Oligomeric structure of the 5-HT_{2A}/mGluR2 heterocomplex: A potential molecular target for new schizophrenia treatments.

J.L. Moreno¹, T. Holloway¹, A. Bortolato³, J.F. Lopez-Gimenez⁴, M. Kurita¹, G. Milligan⁴, S.C. Sealson², M. Filizola³, and J. Gonzalez-Maeso^{1,2}.

Depts. Psychiatry¹, Neurology², and Structural and Chemical Biology³, Mount Sinai School of Medicine, New York, NY 10029. Division of Neuroscience and Molecular Pharmacology⁴, University of Glasgow, Glasgow G12 8QQ, UK.

Monoaminergic neurotransmitters have been the principal focus of schizophrenia research for many decades. Several approaches have also linked the neurotransmitter glutamate to the neurochemical alterations in patients with schizophrenia. Notably, clozapine and other atypical antipsychotics have high affinity for serotonin 5-HT_{2A} receptors (2AR), and metabotropic glutamate receptors 2/3 (mGluR2/3) agonists have shown efficacy in treating schizophrenia. We have identified a functional brain 2AR/mGluR2 complex that may reconcile the monoaminergic and glutamatergic hypothesis of schizophrenia. The next step is to investigate the structure and function of the 2AR/mGluR2 complex, with the ultimate goal of discovering new approaches to the treatment of schizophrenia. We have recently reported that 2AR and mGluR2 form a receptor complex in mouse and human brain, as well as in tissue cultures by co-immunoprecipitation, allosteric binding interaction, bioluminescence resonance energy transfer (BRET) and fluorescence resonance energy transfer (FRET). However, the minimum oligomeric unit required for function is still unknown. We have developed and optimized a sequential three color FRET imaging approach (3-FRET), and demonstrate the expression of 2AR/mGluR2 as higher order oligomers in single cells. We next investigated the specific 2AR and mGluR2 domains responsible for heterocomplex formation. Our results demonstrate that the segment containing transmembrane (TM) domain 4 of mGluR2 is both necessary and sufficient for complex formation with the 2AR. Using a similar approach with 2AR/2CR chimeras our results demonstrate that the TM4 of the 2AR is necessary for heterocomplex formation with mGluR2.

Based on this experimental information, we performed a three-dimensional molecular model of the 2AR/mGluR2 tetramer to be used as a starting point for further simulations. Overall, these data indicate that the 2AR/mGluR2 complex is able to form oligomeric complexes, and that a TM4/TM4 interface is necessary for heterocomplex formation.

Supported by: NIH and NARSAD

Functional Dissociation of the Frontoinsular and Anterior Cingulate Cortices in Empathy for Pain

Xiaosi Gu^{1,2}, Xun Liu¹, Kevin G. Guise^{1,2}, Thomas P. Naidich³, Patrick R. Hof⁴, and Jin Fan^{1,4}

¹Department of Psychiatry, ²Graduate School of Biological Sciences, ³Department of Radiology, and ⁴Department of Neuroscience, Mount Sinai School of Medicine, New York, New York 10029

Introduction: Empathy refers to the ability to understand and echo other people's sensory and emotional states (Gu et al., 2010). The frontoinsular cortex (FI) and the anterior cingulate cortex (ACC) are known to be involved in empathy for others' pain (Singer et al., 2004; Gu and Han, 2007). The FI has been historically considered as a limbic sensory region, and is responsible for polymodal sensory integration (Critchley, 2004; Craig, 2009), while the ACC is known as a limbic motor cortex that participates in voluntary control of multiple domains of behaviors (Bush et al., 2000). Although it is widely accepted that both FI and ACC are co-activated in processing empathy for others' pain, it remains unclear why such structurally distinct regions appear functionally inseparable and what distinct roles they each play in cognitive processes such as empathy for pain. The current study aimed to investigate the specific roles of FI and ACC involved in empathy for others' pain.

Methods: We used functional magnetic resonance imaging (fMRI) to explore the functional dissociation between FI and ACC involved in empathy for pain using a modified empathy for pain task (Gu and Han, 2007). Participants viewed color photographs depicting human body parts (hands or feet) in painful or non-painful situations and performed either pain judgment (painful/non-painful) or laterality judgment (left/right) of the body parts.

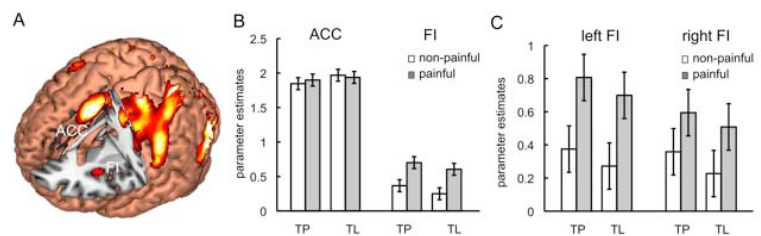
Results and Discussion: Behavioral data (reaction time and accuracy) confirmed that all four experimental conditions had equivalent cognitive load. Neuroimaging data indicated that activation of FI, rather than ACC, showed significant increase for painful compared to non-painful images, regardless of the task requirement. First, FI robustly responded to the sight of others' pain bilaterally, regardless of whether the observer was explicitly asked to evaluate pain. Second, ACC activation did not differentiate between painful and non-painful stimuli, or between pain judgment and laterality judgment; that is, increase in activation due to empathy for pain was significantly greater in FI than in ACC.

Conclusion: We showed for the first time that when cognitive load is carefully matched between painful and non-painful conditions, the FI, but not the ACC, specifically responds to empathy for pain, suggesting a more direct and essential role of FI than ACC in processing empathy for pain. This finding challenges the current consensus that the ACC is indispensable in empathetic responses, and singles out the importance of the FI in empathetic processes (Gu et al., 2010).

Figure 1 Sample stimuli of the experimental stimuli set of 216 digital color photographs showing another person's left or right body hand/foot in painful or non-painful situations. Each stimulus was displayed for 2,500 ms followed by a fixation of 1,500 ms. Subjects were asked to choose between "non-painful" and "painful" for the Task Pain (TP), and "left" and "right" for the Task Laterality (TL) through button press.



Figure 2. ROI analysis of the parameter estimates of ACC and FI for four experimental conditions (TP-non-painful, TP-painful, TL-non-painful, TL-painful). (A) Localization of ACC and FI ROIs derived from activations common to all four experimental conditions. (B) ACC showed comparable activation levels to all four conditions; FI showed significant increased activation for painful compared to non-painful stimuli independent of the task. This ROI-by-stimulus interaction was significant (see Results for details). (C) Responses in left FI and right FI separately. Error bars represent 95% confidence intervals. ACC: anterior cingulate cortex; FI: frontoinsular cortex.



Acknowledgement: This research was supported by National Institute of Health (NIH) Grants R21MH083164 to J.F., by National Center for Research Resources (NCRR) Grant MO1 RR00071, and by The James S. McDonnell Foundation (grant 22002078) to P.R.H. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH or NCRR. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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8**Chronic Cocaine and Stress Engage a Feedback Loop Involving Δ FosB and CaMKII in the Nucleus Accumbens**

A.J. Robison, Vincent Vialou, Matt Wilkinson, Said Kourrich, Miles Collins, Mark Thomas, and Eric Nestler

The transcription factor Δ FosB is stably induced in the nucleus accumbens (NAc) by chronic exposure to stress or 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. We also demonstrate that Δ FosB binds at multiple AP-1 consensus sites within CaMKII promoter regions in a manner regulated by chronic exposure to cocaine or stress. Finally, we provide evidence that Δ FosB may regulate CaMKII mRNA and protein levels in vivo. In combination, these data suggest that CaMKII and Δ FosB engage in a feedback loop as a mechanism for regulating reward circuitry in response to chronic stress or cocaine administration.

9**Cannabinoid-1 and Interleukin 6 receptors synergistically regulate the of re-growth of severed processes in cortical neurons in vitro**

Yana Zorina, Mustafa Siddiq, Marie Filbin and Ravi Iyengar

Department of Pharmacology and System Therapeutics, Mount Sinai School of Medicine

Integration of Go/i-coupled cannabinoid-1 receptor (CB1R) and interleukin 6 receptor (IL-6R) signaling results in enhanced activation of STAT3 and CREB transcription factors, leading to synergistic neurite outgrowth in primary cortical neurons. Since STAT3 and CREB have also been implicated in overcoming myelin inhibition in the context of CNS injury, we hypothesized that activation of CB1R and IL-6R may also promote neurite outgrowth under inhibitory conditions. We found that application of HU-210 (CB1R agonist) and IL-6 at low concentrations to P1 cortical neurons does result in synergistic neurite outgrowth on myelin. Additionally, we used microfluidic chambers that allow separate treatment of the soma and the axonal extensions to mimic axonal injury in vitro. After severing of axonal neurites we tested if treatment at the soma or leading edge was capable of inducing new outgrowth. Somal treatment with HU-210 and IL-6 resulted in synergistic re-growth of processes after they were severed, indicating that signals within the cell body can control growth at distal processes.

(Supported by GM54508. YZ is supported by the Pharmacology Training Grant GM062754)

10**Mechanisms of Gender Differences in Stress-Induced Depressive Behavior**

H. Francisca Ahn, Daniel Christoffel, Sam Golden, Matthew Wilkinson, Weijia Zhang, Li Shen, Scott J. Russo

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Major depressive disorder is one of the largest health burdens to our society with reported occurrences of 3.3% in men and 6% in women. Although women are more likely to suffer from depression and exhibit more severe symptoms, the great majority of studies have used male subjects only. There is also far less known about the molecular mechanisms of gender differences in the development of depressive symptoms. The chronic unpredictable stress (CUS) paradigm is a model used to mimic depressive-like behavior in rodents. Previous findings have shown that females are more sensitive to developing a pro-depressant phenotype following chronic CUS: Females show significantly greater immobility on the forced swim test (FST) and anhedonic response to sucrose compared to males. Although the direct mechanisms driving gender differences are unclear, gonadectomy blocks CUS-induced depressive behavior in females, suggesting that ovarian hormones affect stress hypersensitivity. To further study the underlying the molecular mechanisms of stress hypersensitivity in females, we first examined global gene expression changes in nucleus accumbens (NAc) using RNA sequencing, which provides far better resolution of gene expression than traditional microarray technology. Gene expression levels between males and females differed greatly. Overall, the change in total number of up/down-regulated genes indicated a more robust transcriptional response in males, with 545 genes, than in females, with 301 genes. By comparing the 165 up-regulated genes in females and 287 in males, we find that only 10 genes overlapped. Likewise, of the 136 down-regulated genes in females and 258 in males, only 12 genes overlapped. Therefore, in females, stress may affect gene expression to promote susceptibility, whereas in males there may be an active coping response thereby promoting resilience to the depressive effects of stress. We are currently examining functional groups of genes that differ between males and females to gain greater insight into the mechanisms driving gender differences in stress responses. Future studies are under way to determine the functional role of many of these gene changes in driving stress hypersensitivity in females.

11**Prodynorphin Expression:
A New Perspective on Addiction Vulnerability and Negative Mood States**Sarah Ann R. Anderson¹, Pernilla Fagergren, Ph.D¹, Michael Bannon, Ph.D² and Yasmin L. Hurd, Ph.D¹¹Mount Sinai School of Medicine, New York, New York 10029;² Wayne State University, Detroit, Michigan 48201

Drug addiction is a chronic relapsing disorder characterized by the drive to seek and use drugs despite adverse consequences. Recent studies support that negative emotional states can be motivationally significant in addiction.¹ The emergence of negative mood states, characterized by dysphoria, irritability and anxiety, is strongly associated with withdrawal from drugs of abuse. This negative affect may be propagated by dynorphin, a neuropeptide abundant in the amygdala, a brain region critical for emotional regulation. Such negative mood states may underlie the high comorbidities of addiction with depression, suicide and other mood disorders. Our results demonstrate that cocaine and heroin abusers have significantly altered dynorphin expression in sub-nuclei of the amygdala previously associated with mood disorders.

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. Furthermore, we have shown that there is a significant induction of CEBP δ protein 20 hours after IA training and that C/EBP δ , in both the hippocampus and amygdala, is essential for IA memory consolidation (via antisense knock-down of CEBP δ 48 hrs after training). 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. However, the functional role of C/EBP δ in both nuclear and dendritic compartments remains undetermined. Several transcription factors have been shown to be localized to the dendritic compartment. *In vitro* culture studies have shown that local protein translation and translocation of transcription factors are critical for long-lasting synaptic changes. Therefore we asked whether CEBP δ protein translocates to the nucleus and whether this may be important for long-term memory. Towards this end, protein synthesis dependent late phase LTP (L-LTP) was tetanically induced in hippocampal slices from 3-4 week old Sprague-Dawley rats. Fifteen minutes or 45 minutes after control or L-LTP, hippocampal slices were snap frozen (two 350 μ m slices/condition) or fixed for immunohistochemistry. In fixed slices we observe a qualitative induction of CEBP δ protein, via immunohistochemistry, in L-LTP slices versus control at both time points. To test whether non-nuclear CEBP δ translocates to the nucleus after induction of L-LTP, slices underwent crude subcellular fractionation to enrich either the cytoplasmic or nuclear compartments and then extracts were analyzed via quantitative western blotting. We find that CEBP δ protein is translocated from the cytoplasm to the nucleus 45 minutes after induction of L-LTP. Future studies will address the functional role of CEBP δ protein translocation.

This work was funded by: NIMH-RO1MH063635 and RO1MH074736

13 Molecular convergence between the glucocorticoid receptor- and BDNF-dependent pathways during long-term memory consolidation

*Dhananjay BAMBAH-MUKKU¹, Dillon Y. CHEN¹, Gabriella POLLONINI¹, Cristina M. ALBERINI^{1,2}

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A wealth of literature suggests that de novo protein synthesis and the activation of the evolutionarily conserved cAMP response element binding protein - CCAAT/enhancer binding protein (CREB-C/EBP) pathway are critically required for the formation of long-term memory. However, the upstream signaling pathways and the downstream effectors that are linked to the learning induced protein synthesis and activation of the CREB-C/EBP cascade in vivo during memory consolidation remain unknown. We found that the BDNF and the glucocorticoid receptor (GR) signaling pathways are essential contributors to the initiation of the molecular cascades underlying memory consolidation in the hippocampus. Blocking either the BDNF or the GR signaling pathway in the hippocampus mimics the effects of inhibiting hippocampal protein synthesis on inhibitory avoidance (IA) memory consolidation. In fact, blockade of either hippocampal protein synthesis, BDNF or GR before training results in complete amnesia, whereas the same treatment immediately after training results in partial disruption of memory retention. Moreover, the memory impairment elicited by inhibition of GR is rescued by administration of BDNF suggesting a functional interaction between the two pathways. Finally, we found that this crosstalk is associated with the activation of Ca²⁺/Calmodulin-Dependent Protein Kinase II (CaMKII) and CREB in the dorsal hippocampus. We conclude that a cross-talk between the GR and the BDNF-dependent pathway is critical for memory consolidation and we will test the hypothesis that it significantly contributes to the activation of the underlying protein synthesis and CREB-C/EBP-dependent cascade.

This work was funded by: NIMH Grant R01 MH063635, NIMH Grant R01 MH074736 and NIMH Grant F31 MH081621.

14**Changes in CB₁R expression and activity during neuropathic pain: implications for interactions with DOR**

Ittai Bushlin, Lydia Miller, Lakshmi A. Devi

Dept of Pharmacology and Systems Therapeutics, Mount Sinai School of Medicine

In the United States alone, over two million people suffer chronic and debilitating neuropathic pain as a result of trauma or disease affecting the peripheral or central nervous system. As the treatment of neuropathic pain is difficult and controversial, with only 50% of patients reporting adequate pain relief, novel therapies are in high demand. The endocannabinoid system is a particularly attractive analgesic target because increases in cannabinoid receptor 1 (CB₁R) expression have been identified in animal models of neuropathic pain. This upregulation is believed to be protective and likely mediates the analgesic response to exogenous cannabinoids. These studies have focused on the dorsal root ganglion and spinal cord in several models of neuropathic pain. However, alterations in CB₁R expression and activity have not yet been examined at supraspinal levels, particularly in brain regions involved in pain processing. We find that CB₁R expression and activity are increased in the cortex in a rat model of neuropathic pain.

Changes in CB₁R expression and activity during neuropathic pain could have implications for other receptor systems. Recent studies have demonstrated that CB₁R can functionally interact with other G-protein coupled receptors known to modulate analgesia, including opioid receptors. We have shown in vitro that the delta opioid receptor (DOR) can directly interact with CB₁R, leading to unique downstream signaling. The alterations in CB₁R expression and activity during neuropathic pain may therefore be associated with changes in its interactions with DOR. We find that DOR activity in the cortex of animals experiencing neuropathic pain can be potentiated by a low concentration of CB₁R agonist, and this potentiation correlates with increases in CB₁R expression. This suggests a unique functional interaction between CB₁R and DOR at the cortical level that may result from adaptive increases in CB₁R expression during neuropathic pain.

IB is a trainee in the Integrated Pharmacological Sciences Training Program supported by grant T32GM062754 from NIGMS

15**Expression of Sema7A in the developing rodent brain**

Ioana Carcea and Deanna L Benson

A recent study of the 15q24 microdeletion syndrome identified an atypical deletion in a boy with autism that spans only 15 genes, but shares features with other cases of 15q24 microdeletion syndrome: developmental delay, impaired language and social interaction, sensory dysfunction and recurrent infections. Of the 15 genes identified in this region, SEMA7A stands out as a potentially significant target because data from knockout mice indicate it contributes to the development of certain axon tracts and to immune responses, and the pattern and timing of its mRNA expression in mice suggests it plays a role in synaptogenesis. Nevertheless, very little is known about the function of Sema7A in the brain. We used immunohistochemistry and biochemical approaches to determine the expression of Sema7A protein in the developing brain. Sema7A is expressed in most neuronal structures and is enriched in many axonal tracts like the internal capsule, corpus callosum, cerebral peduncles, anterior commissure etc. Moreover, preliminary data indicate that Sema7A is enriched in the mouse somatosensory neocortex (S1). Within S1, Sema7A concentrates transiently in “barrels”, regions that are critical for the generation of normal sensory processing in rodents, over a time frame that covers the “critical period” for the development of S1 connectivity (a limited interval of high adaptability during development). Additional studies in Sema7A knockout mice will be used to determine the function of the protein in the development of the barrel cortex.

THE GLIA-NEURON INDEX IN THE NEOCORTEX OF CETARTIODACTYLA AND AFROTHERIA: IMPLICATIONS FOR MAMMALIAN BRAIN EVOLUTION

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ABSTRACT

Glial cells (astrocytes and oligodendrocytes) play a fundamental role in many brain functions such as propagation of axon potential, integration of neuronal inputs, modulation of synaptic activity and synaptic strength, modulation of glucose metabolism and extracellular ion concentrations, as well as regulation of synaptogenesis and neurogenesis. Glial cell dysfunction is involved in many neurodegenerative diseases in human. However, the number of glial cells in the brain and its relation to the number of neurons, brain size, and neuronal size remains poorly understood. Although several estimates of the glia-neuron index (GNI) are available for a number of mammals, GNI values from cetaceans are few and were obtained with different techniques from different cortical regions, and are therefore not comparable. We report GNI values obtained by stereologic techniques, in the anterior cingulate (ACC) and primary somatosensory (S1) cortices of cetaceans encompassing a wide range of brain and body sizes including the bottlenose dolphin (*Tursiops truncatus*), the Risso's dolphin (*Grampus griseus*), the harbor porpoise (*Phocoena phocoena*), the beluga whale (*Delphinapterus leucas*), the killer whale (*Orcinus orca*), the dwarf sperm whale (*Kogia simus*), the sperm whale (*Physeter macrocephalus*), the humpback whale (*Megaptera novaeangliae*) and the minke whale (*Balaenoptera acutorostrata*). The brains of the pigmy hippopotamus (*Hexaprotodon liberiensis*), manatee (*Trichechus manatus*), African elephant (*Loxodonta africana*), and rock hyrax (*Procavia capensis*) were used for comparative purposes. Our results show that the thick, neuron-poor layer I influences strongly the GNI in Cetartiodactyla. GNI values vary from 3.54 in the harbor porpoise to 13.19 in the sperm whale. In Afrotheria, the GNI values range from 1.18 in the rock hyrax to 4.93 in the African elephant. Of note, these values from large-brained mammals are generally larger than those reported in various neocortical regions of humans (0.68 to 2.19) and macaque monkeys (0.49 to 1). The large variation of GNI values observed among the species investigated in the present study suggests that the ratio of glial cells to neurons does not represent a conservative feature of the mammalian brain, as previously suggested, and that different values of GNI evolved in different taxa possibly in relation to brain size, neuronal size, and metabolic needs.

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Essential Role for NFκB in Stress-Induced Synaptic Plasticity and Behavior

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Mood and anxiety disorders are devastating afflictions that affect a large portion of the population. The neural mechanisms underlying mood and anxiety disorders are largely unknown, however, a detailed understanding of the neural substrates of these diseases will aid therapeutic development. Chronic social defeat stress in rodents results in a long-lasting behavioral syndrome that models certain aspects of depression-like behavior. The purpose of the current study is to investigate neural correlates that may be responsible for the behavioral phenotypes observed after social defeat. Previous work investigating the effects of stress has largely focused on the limbic circuitry between the amygdala and hippocampus, leaving the role of the nucleus accumbens (NAc), a limbic structure in the ventral striatum that is important in processing the rewarding and emotional salience of stimuli, mostly uncharted.

We first determined whether social stress causes any structural changes in NAc synapses. Analyses of dendritic spine size, shape, and density performed using confocal imaging of Lucifer-Yellow filled cells and semi-automated analysis of selected dendritic segments with NeuronStudio revealed an increase in the total spine density ($p=0.05$) in medium spiny neuron (MSNs) of the NAc shell in susceptible animals. This increase was mostly in immature stubby spines ($p=0.005$). Furthermore, stubby spine density strongly correlates with social interaction (SI) score ($p=0.01$, $r^2=0.65$), suggesting this structural alteration mediates the behavioral phenotype. To further examine the role of synaptic changes associated with social defeat stress we performed an ultra-structural analysis of post-synaptic density (PSD) length. Social defeat induced a shift towards smaller PSDs ($p=0.02$), consistent with our spine analysis and PSD length also strongly correlated with SI score ($p=0.002$, $r^2=0.58$).

In parallel with these structural changes, we have observed significant increases in nuclear factor kappa B (NFκB) in NAc after social defeat stress. While little is known about the role of this transcription factor in structural plasticity, activation of NFκB signaling pathways may be responsible for these changes in neuronal morphology. Furthermore, using a herpes simplex viral vector to express mutant Inhibitor of kappa Kinase to either activate or inhibit NFκB signaling (IKKca & IKKdn, respectively) we find that activation of this pathway is both necessary and sufficient to induce the susceptibility phenotype. Future studies to examine whether these manipulations also block stress-induced changes in neuronal morphology are currently underway.

18 Prenatal cannabis exposure alters the epigenetic regulation of the dopamine receptor

D2 gene in the nucleus accumbens

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A dramatic shift in our recent understanding of the neurobiology of mental disorders has acknowledged that drug addiction is a brain disease that is strongly interrelated with neurodevelopment. Marijuana (*Cannabis sativa*) is the illicit drug most commonly abused by pregnant women and prenatal cannabis exposure has been linked with increased susceptibility to behavioral disturbances including drug abuse. The neurobiology underlying this long-term effect is, however, unknown. Various lines of evidence from our group and others suggest that the neurodevelopmental effects of prenatal cannabis exposure target the ‘indirect’ striatopallidal neuronal circuit. An important component of this pathway, the dopamine D2 receptor (D₂R), has been strongly implicated in addiction risk. We now present evidence for disrupted D₂R regulation in the ventral striatum of midgestational human fetal and rat subjects with maternal cannabis exposure. A selective reduction in mRNA levels of the dopamine receptor D2 gene (*Drd2*) was observed in the nucleus accumbens, but not dorsal striatum, of fetal subjects with in utero cannabis exposure, and this effect persisted into adulthood in rats exposed to Δ^9 -tetrahydrocannabinol (THC) prenatally. We subsequently began to explore the nature of gene regulation mechanisms that could maintain aberrant *Drd2* expression and neuronal processing as a result of developmental cannabis exposure. The epigenome is known to be influenced by environment and thus is a highly relevant biological candidate to mediate such effect. To investigate this possibility, we performed chromatin immunoprecipitation in the adult rat NAc to analyze dimethylation of lysine 9 on histone H3 (2meH3K9), a chromatin modification with a well-known role in developmental gene silencing. Experiments using rats treated with THC *in utero* show that the observed reduction of *Drd2* expression is related to impairment of 2meH3K9 at a specific genomic region upstream of the *Drd2* transcription start site. Taken together, these results suggest that maternal cannabis use alters the developmental regulation of mesolimbic D₂R in the offspring through epigenetic mechanisms that regulate repressive histone lysine methylation, and this may contribute to increased addiction vulnerability later in life.

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19 **Optogenetic stimulation of the medial prefrontal cortex has antidepressant-like effects**

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Human deep brain stimulation and imaging studies have highlighted a key role for the prefrontal cortex in clinical depression, however, it remains unknown whether excitation or inhibition of prefrontal cortical neuronal activity is associated with antidepressant responses. The objective of the current series of experiments is to examine the relationship between chronic stress-induced molecular modifications of the mPFC and depressive-like behaviors in mice. The functional activity of infralimbic and prelimbic cells were inferred from zif268 expression, CREB activity, and c-fos expression after chronic social defeat stress. Here, we report that cellular indicators of functional activity, including the immediate early gene zif268 and the transcription factor creb, are reduced in prefrontal cortex of depressed human brains obtained postmortem and after chronic social stress in mice. Deficits in mPFC functional activity persist long after social stress, and these deficits are corrected via stimulation of the mPFC using virally-mediated expression of channel rhodopsin 2 (ChR2) which when activated with a blue laser (470nm) will result in neuronal firing. Laser stimulations mimicking patterns of mPFC “burst” firing, not only restored functional activity, but also corrected behaviors impaired by social stress, indicating strong antidepressant-like effects of mPFC stimulation.

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20 **VGF Deficiency Perturbs the Storage of Chromogranin B in Adrenal Chromaffin Granules and Decreases Granule Size**

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Secretion of hormones, growth factors, and neuropeptides from endocrine and neuronal cells is a highly regulated process, but the molecular mechanisms controlling the biogenesis of dense core secretory granules (DCGs) is not well understood. It is thought to involve interaction of multiple proteins that are stored in DCGs, including the granin proteins chromogranin A (CgA) and chromogranin B (CgB). VGF, a member of the extended granin protein family, is selectively expressed in neural and endocrine tissues, including adrenal medulla, and is processed into bioactive peptides and released via the regulated secretory pathway.

Genetic ablation and peptide injection studies indicate that VGF plays a critical role in the regulation of energy balance, memory and depressive behavior. Here we present new evidence that VGF gene ablation affects DCG biogenesis. Analysis of VGF knockout mice reveals a decrease in DCG size, in adrenal medulla, with enlargement in the volume around the dense core. In addition, levels of other DCG components are affected. We observed a significant decrease of CgB, but not CgA or SgII, and a slight increase in SgIII protein levels in VGF KO mouse adrenal. These may reflect compensatory changes resulting from VGF deficiency, but could also be the result of altered protein:protein binding in the DCG lumen. Consistent with the latter, co-immunoprecipitation experiments suggest an interaction between VGF and CgB in PC12 pheochromocytoma cells. Other granins that interact with VGF in vitro and in vivo are currently under investigation. These data suggest an important new role for VGF in DCG biogenesis, potentially regulating hormone, growth factor, and/or neuropeptide secretion.

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21 Beclin-1 mediated autophagy regulates the accumulation of hyperphosphorylated tau

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The autophagy pathway is the major degradation pathway of the cell for long-lived proteins and organelles. Dysfunction of autophagy has been linked to several neurodegenerative disorders that are associated with an accumulation of misfolded protein aggregates. Alzheimer's disease (AD), the most common neurodegenerative disorder, is characterized by two aggregate forms, tau tangles formed from hyperphosphorylated tau and amyloid- β (A β) plaques. Autophagy has been linked to AD pathogenesis through its merger with the endosomal-lysosomal system, which has been shown to play a role in the formation of the latter A β plaque. However, the precise role of autophagy in AD pathogenesis is still under contention. One hypothesis is that aberrant autophagy induction results in an accumulation of autophagic vacuoles containing A β and the components necessary for its generation while other evidence points to impaired autophagic clearance or even an overall reduction in autophagic activity playing a role in AD pathogenesis. A recent study demonstrated that the autophagy protein beclin 1 exhibited decreased expression in affected brain regions of AD patients in comparison to age-matched controls. In the present study, we show that reduced expression of beclin 1 in both beclin 1 heterozygous and conditional knockout mouse models results in impaired autophagy and increased levels of phosphorylated tau in the brain in an age-dependent manner. Conversely, overexpression of beclin 1 in a transgenic mouse line results in decreased tau phosphorylation. Our ongoing study reveals an inverse correlation of beclin 1 expression with tau hyperphosphorylation, giving further support to the hypothesis that impaired autophagy plays a critical role in AD pathogenesis.

22 Memory reconsolidation mediates memory strengthening in a temporally limited fashion

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Newly learned information initially exists in a labile state, and over time stabilizes into a long-term memory by a consolidation process that requires RNA and protein synthesis. Established memories can again become transiently sensitive to disruption if reactivated, for example by a non-reinforced exposure, and require a process of reconsolidation in order to persist. The reason/s why memory undergoes reconsolidation still remain to be elucidated. In this study, we tested the hypothesis that reconsolidation mediates memory strengthening. Using the inhibitory avoidance (IA) paradigm in rats, we found that multiple reactivations by context exposures resulted in a significant enhancement of IA memory retention. Importantly, memory retention could be disrupted if protein synthesis was inhibited after each reactivation in younger (one week old) but not in older (3 weeks old) memories, suggesting that the passage of time is also a major contributor to memory stabilization and strengthening. Furthermore, multiple reactivations during the first week after training lead to increased memory retention tested 2 months later suggesting that reconsolidation contributes to prevent forgetting. However, a 28 day-old memory, when exposed to the same multiple reactivation protocol undergoes extinction, suggesting that time changes the features of the memory and the response to reactivation events. In agreement with previous work from other laboratories, we found that the duration of the retrieval exposure temporally affects the protein synthesis dependent phase of reconsolidation. Hence, we conclude that memory reconsolidation mediates memory strengthening and prevents forgetting in a temporally limited fashion.

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23**Behavioral responses that require the 5-HT_{2A}/mGluR2 complex
in mouse models of schizophrenia.**

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Schizophrenia is a chronic mental illness affecting nearly 1% of the population. The causes of schizophrenia and the mechanism of action of antipsychotics are unknown. The neurotransmitters glutamate and serotonin both have been the target of considerable attention regarding psychosis and antipsychotic drug development. Thus clozapine and other atypical antipsychotics have high affinity for 5-HT_{2A} receptors (2AR), and metabotropic receptors 2/3 (mGluR2/3) agonists have recently shown efficacy in treating schizophrenia. We have identified a novel functional 2AR/mGluR2 complex that may be responsible for some of the molecular mechanisms responsible for psychosis. Psychotomimetic drugs such as phencyclidine (PCP) and lysergic acid diethylamide (LSD) induce schizophrenia-like psychosis in humans, and represent in mouse a behavioral model of schizophrenia and psychosis. We have previously demonstrated that head-twitch is reliably and robustly elicited by LSD-like drugs, and is absent in 2AR null-mutant mice. Interestingly, the head-twitch response induced by the hallucinogenic 2AR agonist DOI was significantly lower in mGluR2 null-mutant mice. Activation of mGluR2, but not mGluR3, has been found to reduce the behavioral stereotypy and hyperlocomotion produced by PCP-like drugs. The locomotor activity elicited by the PCP-like drug MK801 in wild-type and 2AR null-mutant mice was indistinguishable. Notably, the MK801-stimulated locomotor activity was significantly attenuated by the mGluR2 agonist LY379268, but it was not affected in 2AR-null mutant mice. Based on these results, we propose that the psychedelic effects of 2AR agonists, as well as the antipsychotic properties of mGluR2 agonists, may be due, in part, to the signaling crosstalk resulting from their activity at the 2AR/mGluR2 complex in cortical neurons.

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24**Visualization of Specific Protein Degradation at Hippocampal Synapses**

Kuangfu Hsiao

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Developmental changes in synaptic proteome are locally regulated by both protein degradation and synthesis; however, the importance of regulated protein turnovers is just beginning to dawn. Here, I hypothesize that the neuronal plasticity related protein calcium/calmodulin-dependent protein kinase II (CaMKII) is not only rapidly synthesized at synapses and is also rapidly degraded by the proteasome in an N-terminal (Nt) acetylation mediated, Doa10 ubiquitin ligase targeted, so called N-end rule pathway. We designed a degradation reporter to visualize synaptic CaMKII whose spatiotemporal degradation is regulated by N-terminal methionine aminopeptidases (MetAP2), Nt-acetylase (ARD1), and Doa10. When N-end rule pathway is suppressed, a set of synaptic proteins, specifically CaMKII, are rapidly accumulated in synaptic compartment. I also hypothesize that the requirement of N-end rule pathway is associated with the dependence of local protein synthesis at labile synapses. I will examine the necessity of N-end rule pathway during synapse maturation.

HDAC1 regulates behavioral responses to cocaine and chromatin dynamics in the nucleus accumbens

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Increasing evidence suggests that changes in histone acetylation and histone methylation in the nucleus accumbens (NAc) and other brain reward regions mediate drug-induced plasticity by facilitating or repressing gene expression. Histone deacetylases (HDACs) are a family of enzymes that repress gene expression by removing acetyl groups from histones. Systemic, or intra-NAc, treatment with non-specific HDAC inhibitors increases behavioral responses to cocaine, while overexpression of certain class II HDACs blocks the rewarding effects of the drug.

Using viral-mediated knockdown of HDAC1, a class I HDAC, in floxed HDAC1 mice, or infusion of MS275, an inhibitor that preferentially targets HDAC1, into the NAc, we demonstrate a specific role for this enzyme in behavioral responses to cocaine. Specifically, we show that short-term HDAC inhibition increases the locomotor-activating effects of cocaine following a challenge dose, whereas prolonged HDAC inhibition or viral knockdown of HDAC1 abolishes the locomotor-activating effects of the drug. Furthermore, we demonstrate that while both short-term and prolonged HDAC inhibition in the NAc increase activating histone acetylation, only prolonged HDAC inhibition produces a concomitant increase in repressive histone methylation and increased gene expression of enzymes responsible for histone methylation.

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26 Cell Type Specific Loss of BDNF Signaling Mimics Optogenetic Control of Cocaine Reward

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The nucleus accumbens (NAc) is a key mediator of cocaine reward, but the distinct roles of the two subpopulations of NAc projection neurons are poorly understood. The projection neurons of the NAc and dorsal striatum (dStr) are medium spiny neurons (MSNs) and they are differentiated into two subpopulations based on their projections and their differential expression of G-protein coupled receptors including dopamine receptors, D1 receptor and D2 receptor. We show that deletion of TrkB, the brain-derived neurotrophic factor (BDNF) receptor, selectively from D1+ or D2+ MSNs oppositely affects cocaine reward. Furthermore, after exposure to cocaine we observe opposite regulation of c-Fos in D1+ and D2+ MSNs in the NAc when TrkB is deleted in each population. Given that the attenuation of cocaine reward triggered by loss of TrkB from D2+ neurons is associated uniquely with induction of c-Fos, a marker of neuronal activity, we used optogenetic tools to control selectively the firing rate of D1+ and D2+ nucleus accumbens neurons and studied consequent effects on cocaine reward. We show that activation of D2+ neurons, mimicking the loss of TrkB, dramatically suppresses cocaine reward, with opposite effects induced by activation of D1+ neurons. These results provide novel insight into the molecular control of D1+ and D2+ neuronal activity as well as the circuit level contribution of these cell types to cocaine reward.

27 **Generation of induced pluripotent stem cells from cobalamin C deficient fibroblasts for the functional studies of oligodendrocyte with defective vitamin B12 metabolism**

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Abstract

Defects in CblC, the most common type of inborn error of metabolism of vitamin B12 (cobalamin), cause methylmalonyl acidemia combined with homocysteinuria. Prominent neurological symptoms manifest as feeding problems in early infancy, hypotonia and progressive neural degeneration. Even with the supplementary treatment, the disease continues to progress and possibly lead to premature death. The gene locus of CblC has been recently mapped on chromosome 1, but the exact mechanism underlying the pathology in the central nervous system is not clearly understood.

One of the manifestations of cblC mutations in patients is axonal demyelination, which can in part explain the neurological symptoms. However it is unclear whether the lack of myelin around the axons is the result of defective differentiation of the oligodendrocytes or death of these cells. In this study, we aim to generate induced pluripotent stem cells (iPSCs) from cblC patients' fibroblasts and generate oligodendrocyte progenitors. This will allow us to test the myelin generating capacities of cblC patients compared to normal controls, which will be the model system for the functional study of CblC in myelination.

To establish a standardized protocol of differentiation of established iPSCs into oligodendrocytes, human embryonic stem cells H9 have been used. H9 hES cells were first allowed to form cell clusters, also known as embryoid bodies, by withdrawing the bFGF, which is required to maintain the pluripotency. Embryoid bodies were reattached to the laminin coated surface for further differentiation in the presence of N2 supplement with retinoic acid until neural tube-like rosette structures were formed, which express neural stem cell marker, Pax6. These cells were further matured into oligodendrocyte progenitors in the presence of sonic hedgehog characterized by the expression of Olig2 and Nkx2.2. These cells were also tested positive for the oligodendrocyte progenitor surface markers, A2B5 and NG2.

Here we are also reporting the work in progress in the generation of iPSCs from cblC patients-derived fibroblasts as well as from age- and gender-matched controls. We have successfully generated iPSCs from the control group using retroviruses expressing hOct4, hSox2, hKlf4 and hMyc as previously reported. Selection of iPSCs has been based on morphological criteria of resemblance of human embryonic stem cells for cellular and colony identification. Selected iPSC colonies were tested for expression of hES markers using immunocytochemistry and quantitative realtime PCR.

Initially, CblC-deficient fibroblasts failed to generate any iPSC colonies in the same condition used for control fibroblasts. This suggests that physiology of cblC itself may be hindering with reprogramming of fibroblasts into iPSCs. However, in the presence of high concentration of hydroxo-cobalamin, we were able to generate initial iPSC-appearing colonies and we are in the process of characterizing their reprogrammed pluripotency.

28 **Group II metabotropic glutamate receptor stimulation triggers production and release of Alzheimer's amyloid β 42 from isolated intact nerve terminals**

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ABSTRACT

Aberrant accumulation of amyloid beta ($A\beta$) oligomers may underlie the cognitive failure of Alzheimer's disease (AD). All species of $A\beta$ peptides are produced physiologically during normal brain activity. Therefore, elucidation of mechanisms that interconnect excitatory glutamatergic neurotransmission, synaptic amyloid precursor protein (APP) processing and production of its metabolite $A\beta$, may reveal synapse-specific strategies for suppressing the pathological accumulation of $A\beta$ oligomers and fibrils that characterize AD. In order to study synaptic APP processing, we used isolated intact nerve terminals (cortical synaptoneuroosomes) from TgCRND8 mice, which express a human APP with familial AD mutations. Potassium chloride depolarization caused sustained release from synaptoneuroosomes of $A\beta_{42}$ as well as $A\beta_{40}$ and appeared to co-activate α -, β - and γ -secretases, which are known to generate a family of released peptides, including $A\beta_{40}$ and $A\beta_{42}$. Stimulation of postsynaptic Group I mGluRs with DHPG induced a rapid accumulation of APP carboxy terminal fragments (CTFs) in the synaptoneuroosomes, a family of membrane-bound intermediates generated from APP metabolized by α - and β -secretases. Following stimulation with the Group II mGluR agonist DCG-IV, levels of APP CTFs in the synaptoneuroosomes initially increased, but then returned to baseline by 10 minutes after stimulation. This APP CTF degradation phase was accompanied by sustained accumulation of $A\beta_{42}$ in the releasate, which was blocked by the Group II mGluR antagonist LY341495. These data suggest that Group II mGluR may trigger synaptic activation of all three secretases and that suppression of Group II mGluR signaling may be a therapeutic strategy for selectively reducing synaptic generation of $A\beta_{42}$.

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29 Role of BDNF in the VTA in regulating molecular and behavioral responses to morphine

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Repeated exposure to opiates produces long-term adaptive biochemical and morphological alterations in the mesolimbic dopamine system, which comprises dopaminergic neurons in the ventral tegmental area (VTA) and their projections to the nucleus accumbens (NAc) and other forebrain structures. Signaling of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), in the mesolimbic system has been suggested as a critical mediator of addiction-related changes. However, functional interactions between morphine and BDNF signaling have not been well studied.

In the present study, we found that localized knockdown of BDNF or of TrkB in the VTA, using localized viral-mediated Cre recombinase expression in floxed BDNF or floxed TrkB mice, enhances morphine reward as measured by place conditioning. In contrast, localized BDNF or TrkB knockdown in the NAc had no significant influence on morphine reward. These data suggest that BDNF signaling in VTA, but not NAc, has an antagonizing effect on the behavioral actions of morphine.

To understand how BDNF is regulated in the VTA by morphine treatment, we analyzed BDNF mRNA and chromatin modifications using RT-PCR and chromatin immunoprecipitation. We found that BDNF mRNA levels were decreased in the VTA by chronic morphine treatment and that this reduction was accompanied by chromatin modifications such as reduction in poly-acetylation and in trimethylation of Lys 4 of histone 3 at the *Bdnf* promoters P1 and P2. In addition, chronic morphine decreased the binding of phospho-CREB at the *Bdnf* P3 and P4 promoters. In contrast, chronic morphine increased MeCP2 binding to CpG islands in *Bdnf* P2 and P3 regions in the VTA, changes not associated with morphine-induced changes in DNA methylation of these promoter regions, suggesting MeCP2 bindings to un-methylated DNA.

To investigate the role of BDNF signaling in the VTA in regulating genome-wide changes in gene expression in the NAc, we performed microarray analysis on NAc tissue from mice in which BDNF was knocked down in the VTA. We identified clusters of genes that are regulated by morphine, but whose regulation is abolished by knockdown of VTA BDNF (e.g., *Ptpn2*). In contrast, other genes, which are not regulated by morphine in normal animals, show such regulation upon knockdown of VTA BDNF (e.g., *Zbtb16*). Still other genes display common regulation regardless of BDNF knockdown (e.g., *Camk1g*).

Together, these findings implicate the BDNF signaling in the VTA as a critical regulator of chronic morphine action at the transcriptional, epigenetic, behavioral, and cellular levels.

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Diabetes-linked SORCS1, a γ -secretase substrate and regulator of Alzheimer's APP metabolism, forms complexes with multiple Alzheimer's-linked proteins (APP, PS1, BACE, VPS35, and SORL1)

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Abstract:

Background:

SORL1 (LR11/SorLA) and SORCS1 belong to the sortilin family of vacuolar protein sorting-10 (Vps10) domain containing receptors. SORL1 and SORCS1 are genetically associated with Alzheimer's disease (AD), and SORL1 expression is decreased in the brains of patients suffering from mild cognitive impairment and AD. SORCS1 has additionally been genetically associated with both Type 1 Diabetes (T1D) and Type 2 Diabetes Mellitus (T2DM) and its complications, representing a common factor that might explain why AD and DM frequently coexist. In this current study we sought to determine the role of SORCS1 in APP processing.

Methods:

APP metabolite generation was analyzed in brains of male and female Sorcs1-deficient mice. Levels of α and β C-terminal fragments (CTF) were analyzed by western blot analysis using pAb 369 directed against the C-terminus of APP. Levels of $A\beta$ [x-40] and $A\beta$ [x-42] were analyzed by sandwich ELISA. In an effort to identify key SORCS1-modulated steps in APP processing, co-immunoprecipitations were performed to identify complexes between SORCS1 and key components of the APP processing and trafficking machinery.

Results:

Here, we show that levels of Alzheimer's amyloid precursor protein (APP) metabolites are altered in Sorcs1-deficient mice. Increases in α -CTF (20% p=0.01) and β -CTF (30% p=0.025) were seen in the brains of Sorcs1-deficient mice compared to wild type littermates. Interestingly in the brains of female Sorcs1-deficient mice a strong trend towards increased $A\beta$ x-42 (p=0.07) was observed. We additionally investigated the possibility that SORCS1 might directly interact with components of the APP processing apparatus. We identified a tripartite complex consisting of APP, SORL1 and SORCS1. We also recovered complexes linking SORCS1 to several other components of the APP processing apparatus, including SORCS1:VPS35 retromer complexes, SORCS1: β -site APP cleaving enzyme complexes, and SORCS1:presenilin 1 complexes.

Conclusions:

Taken together, these data demonstrate the existence of a complicated set of VPS10-protein modulated protein-protein interactions with APP. This represents the first evidence of the cell biological changes that might result from the interaction of AD and DM-related protein SORCS1. We speculate that these interactions may, in some cases, contribute to the increased risk for AD that is associated with DM.

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Epigenetic Modifiers Are Necessary but Not Sufficient for the Reprogramming of Non-Myelinating into Myelin Gene-expressing Cells

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Abstract

Background:

Modifications on specific histone residues (termed “histone code”), together with DNA methylation, play an essential role in the specific lineage choices during cell differentiation. Previous studies have shown the importance of histone deacetylation during the differentiation of progenitors into myelinating oligodendrocytes, however it is not clear whether manipulation of these modifications is sufficient to profoundly affect gene expression in unrelated cell types and switch cell identity. Despite the application of epigenetic modifiers in reprogramming fibroblasts into stem cells or neuron-like cells, whether global epigenetic modification might be helpful for the trans-differentiation of fibroblasts into oligodendrocyte-like cells has not been tested.

Methodology/Principal Findings:

We performed the first characterization of the histone code established on the myelin genes during the progression of primary oligodendrocyte progenitors along the lineage, and compared it with the one detected in NIH3T3 fibroblasts and myelinating cell lines (Oli neu). Using chromatin immunoprecipitation (ChIP) analysis and antibodies for histone markers of activation (me3H3K4 and AcH3) or repression (me3H3K9 and me3H3K27), we demonstrated the presence of a cell-specific histone code on the highly conserved regions of Mbp and Mag gene. Myelin genes in fibroblasts were characterized by the presence of repressive histone modifications. In an attempt to revert these changes associated with a non-permissive conformation of chromatin, we treated fibroblast lines with the DNA demethylating agent 5-azadeoxycytidine (5-AzaC) alone or in combination with inhibitors of histone deacetylases (TSA) or sirtuins (sirtinol). Combination treatments resulted in rapid induction of endogenous myelin gene expression in fibroblasts, although not to the levels detected in myelinating cells. This was associated with concomitant upregulation of positive and negative regulators of myelin gene expression and genes affecting pluripotency. Furthermore, combination of epigenetic modifier agents followed by over-expression of oligodendrocyte-specific transcription factors (TFs) in fibroblasts was not able to further induce the expression of myelin genes.

Conclusions/Significance:

These results suggest that global epigenetic manipulation is necessary but not sufficient to trans-differentiate a fibroblast into a myelinating cell.

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32**Single spike induced alterations of intracellular calcium in a sensory neuron**B. Ch. Ludwar¹, C. G. Evans^{1, 2}, E. C. Cropper¹;¹ Dept. of Neuroscience, Mt. Sinai School of Medicine, ²Phase Five Communications, New York, NY

We study sensorimotor transmission and have described two distinct regulatory mechanisms in our model system, which consists of the Aplysia mechanoafferent B21 and a follower motoneuron B8. The two mechanisms potentiate synaptic transmission, and are induced by increases in the presynaptic membrane potential. We recently showed that one mechanism is calcium dependent. When a dihydropyridine sensitive calcium current is pharmacologically blocked, B21 induced PSPs are virtually eliminated in B8. This dihydropyridine sensitive calcium current is activated when B21 is held at depolarized potentials below the spike threshold and its induction results in a widespread increase in the intracellular free calcium concentration. The current study focuses on changes in calcium concentration induced by spiking.

To determine whether changes in the intracellular calcium concentration can be detected during individual spikes, neuron B21 was injected with the calcium indicator dye Calcium Orange and spikes were triggered by brief mechanical stimulation of the subradula tissue. Spike induced increases in calcium fluorescence were observed in the medial, somatic, and lateral region of B21. These increases were larger at more depolarized potentials, and the effects of membrane potential were graded. This suggests that potentiating effects of depolarization on B21-B8 synaptic transmission may in part be mediated by effects of holding potential on calcium influx that occurs during spiking. Interestingly, spike induced increases in calcium fluorescence had a relatively long relaxation rate, which suggested that they would summate if multiple spikes were triggered. To study summation, we peripherally activated B21 with a physiologically relevant stimulus that induces burst of spikes (stretch of the subradula tissue). Summation was observed when bursts of spikes were repeatedly generated at inter-cycle intervals typical for feeding motor programs. This type of summation could therefore constitute a mechanism for progressive enhancement of sensorimotor transmission as B21 is repeatedly activated during an ongoing motor program.

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33**G9a regulates cocaine-induced behavioral and transcriptional plasticity in a cell-type specific manner**Ian Maze¹, Anne Schaefer², David M. Dietz¹, Herbert E. Covington, III¹, Srihari Sampath³, Alexander Tarakhovskiy³, Paul Greengard², and Eric J. Nestler¹¹Fishberg Department of Neuroscience, Mount Sinai School of Medicine, New York, NY; ²Laboratory of Molecular and Cellular Neuroscience, ³Laboratory of Lymphocyte Signaling, The Rockefeller University, New York, NY

Chronic cocaine-induced alterations in gene expression cause cell-type specific 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 cell-type specific cocaine-induced behavioral and transcriptional plasticity. Using G9a^{fl/fl} mice expressing either a Dopamine-1- or Dopamine-2-receptor (Drd1 or Drd2) neuronal specific Cre-recombinase, we limited G9a deficiency to either Drd1- or Drd2-expressing neurons in the limbic forebrain. Through such cell-type specific deletion of G9a, we demonstrate that G9a activity in response to cocaine promotes altered cocaine sensitivity and reward in a cell-type specific manner. G9a^{fl/fl}; Drd1- and Drd2-Cre expressing animals were further crossed to bacterial artificial chromosome (BAC) transgenic mice specifically expressing an EGFP-tagged ribosomal protein in either Drd1- or Drd2-expressing neurons. Using genetically targeted translating ribosome affinity purification (TRAP), we further examined how G9a-mediated gene transcription is affected following chronic cocaine exposure in Drd1- and Drd2-expressing striatal neurons. These data indicate an essential role for G9a in the cell-type specific regulation of behavioral and transcriptional plasticity following chronic cocaine administration.

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34 Alterations in mTOR signaling and excitability of dopamine neurons in the ventral tegmental area after chronic opiate exposure.

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Given the escalating use and abuse of pain-relieving opiate drugs in both the adult and adolescent U.S. population, our laboratory has been interested in identifying neuroadaptations that occur in response to chronic opiate exposure. To this end, we have shown that chronic morphine administration or heroin self-administration decreases the size of dopaminergic (DA) cells within the ventral tegmental area (VTA), a critical brain region in the development of addiction. Previous studies indicated that this morphological change, as well as the associated behavioral phenotype of reward tolerance, is mediated by decreased neurotrophic factor signaling, specifically downregulation of Akt activity. Our current goals are to elucidate the signaling mechanisms downstream of Akt as well as the precipitating factors that lead to decreased cell size and reward tolerance. We have evidence that signaling of a well-established cell growth pathway, mammalian target of rapamycin (mTOR), is altered in the VTA in response to chronic opiate treatment. We observed, via western blot, increased phosphorylation of two substrates of mTOR complex 1 (mTORC1), p70S6K and 4EBP, and decreased phosphorylation of two mTOR complex 2 (mTORC2) substrates, Akt and PKC α . Administration of rapamycin did not block morphine-induced morphological changes, suggesting that the decreased mTORC2 activity, not the increased mTORC1 activity, is mediating the cell size changes. We also have evidence that these biochemical changes may be occurring specifically in DA neurons in the VTA as we observed an increase in the proportion of dual-labeled phospho-S6 and tyrosine hydroxylase (TH) cells using immunohistochemistry; no difference in phospho-S6 signal was observed in TH-negative cells in response to morphine treatment. We have begun examining the electrophysiological activity of VTA DA neurons in an effort to understand how morphine may be mediating changes in neurotrophic and mTOR signaling. Using our chronic morphine paradigm that produces biochemical changes, we observe a significant increase in the firing rate of DA neurons. Additionally, using viral-mediated overexpression of either wild-type or dominant-negative potassium channels in the VTA, which inhibit or activate neuronal activity, we are able to increase and decrease DA cell size, respectively. These findings suggest that morphine-induced changes in firing rate are sufficient to induce morphological changes in the VTA. Current studies are examining whether the change in firing rate is necessary to induce the biochemical, morphological, and behavioral changes induced by chronic morphine.

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35 **Gene Expression Signature in the Nucleus Accumbens of Human Heroin Abusers**

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Nucleus Accumbens (NA) hosts cluster of neurons that are believed to play a key role in psychological states such as pleasure, craving, and reward. Understanding aberrant changes in gene expression patterns in NA due to chronic drug abuse is an important step toward discovering the complex molecular mechanisms involved in drug addiction, and consequently to suggest promising targets. In this study we sought to identify the genes that are differentially expressed in NA of PMBs between samples from chronic Heroin abusers vs. controls. Samples were collected from a homogenous population of Heroin addicted males (19 samples) and females (3 samples) as well as individuals with no history of addiction to Heroin (22 men and 5 women). Preliminary microarray analysis suggests that over 300 genes significantly changed between the two groups. Amongst them 54 genes showed modest upregulation ($> \sim 1.4$ fold, $p < 0.05$) and 94 genes displayed at least 0.7 fold decrease in expression ($p < 0.05$). Enrichment analysis revealed that targets of mir-10, mir-543, and mir-200 are overrepresented in the up-regulated genes ($p < 0.01$). We also found that two signaling pathways that are involved in calcium regulation were overrepresented in the up-regulated gene set ($p < 1-5$). These pathways are known to contribute to long-term potentiation (LTP). The down-regulated genes are associated with cell-cycle and apoptosis pathways ($p < 0.01$). We are currently working to further elaborate on these effects as well as to identify genes that can serve as biomarkers for long term Heroin abuse.

36 **Hypothalamic FKBP5 is induced by fasting and leptin deficiency and elevated expression promotes obese phenotypes.**

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To discover genes that might play a role in maintaining obesity, we carried out a microarray screen for genes induced by a 48-hour fast in male C57Bl/6J mouse ventromedial hypothalamus (VMH), which includes the VMN and arcuate nucleus. One such gene was FKBP5 (FK506 binding protein 5; Locus NP_034350), whose induction by fasting and by leptin deficiency mice in VMH we corroborated by qPCR. Subsequent analysis demonstrated that a 48-hour fast induces FKBP5 in VMN, arcuate, and PVN, of both mice and rats. To assess if hypothalamic FKBP5 plays a role in promoting obesity, the gene was transferred to the VMH via an AAV vector. Within 2 weeks after FKBP5 transfer, mice on a high-fat diet exhibited elevated body weight, without hyperphagia, relative to mice receiving the GFP gene. Body weight remained elevated for more than 8 weeks, and was associated with impaired glucose tolerance. These studies suggest that elevated hypothalamic FKBP5 promotes obese phenotypes, possibly by blocking glucocorticoid negative feedback effect.

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Differential role of Δ FosB and $\Delta 2\Delta$ FosB in response to stress and drugs of abuse

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The *fosB* gene has an exonic intron in exon 4/5, which allows alternative splicing and the encoding of two distinct mRNAs, FosB and Δ FosB. The latter is a truncated form of FosB, which lacks 101 aa at its C-terminal transactivation domain. In addition, the *fosB* gene has three alternative translation initiation sites in exon 2. This means that, together, the *fosB* gene has the potential to express eight protein products, FosB and its 3 N-terminal truncations, termed $\Delta 1$ FosB, $\Delta 2$ FosB, and $\Delta 3$ FosB, plus Δ FosB and its 3 N-terminal truncations, $\Delta 1\Delta$ FosB, $\Delta 2\Delta$ FosB, and $\Delta 3\Delta$ FosB.

Due to translational efficiency, the main products of the *fosB* gene in brain are FosB, Δ FosB, and $\Delta 2\Delta$ FosB; the latter is a truncated form of Δ FosB, which lacks 78 aa at its N-terminus. Importantly, both Δ FosB and $\Delta 2\Delta$ FosB accumulate in response to repeated or prolonged stimuli due to their extraordinary stability. We have reported that Δ FosB accumulates in a region-specific manner in brain after chronic exposure to several types of stress, electroconvulsive seizures, or drugs of abuse, and that Δ FosB, acting in the nucleus accumbens—a key brain reward region, enhances drug reward and promotes antidepressant-like responses. We have also shown that Δ FosB can act as either a transcriptional activator or repressor both *in vitro* and *in vivo*. However, in most cases, these activities were observed under conditions when both Δ FosB and $\Delta 2\Delta$ FosB accumulate. This is because endogenous and transgenic expressing mRNA constructs of Δ FosB permit $\Delta 2\Delta$ FosB expression.

The goal of the present study was to determine whether the previously reported activities of Δ FosB are dependent on Δ FosB *per se* or also on $\Delta 2\Delta$ FosB. To accomplish this goal, we made adeno-associated virus (AAV) vectors that express Δ FosB^{BM79LM99L} (which prevents N-terminal truncations to $\Delta 2\Delta$ FosB or $\Delta 3\Delta$ FosB), or $\Delta 2\Delta$ FosB, or FosB. The vectors express Venus after an IRES2, and AAV-IRES2-Venus served as a control. We employed a broad behavioral battery, including social defeat and cocaine conditioned place preference, after injection of these vectors into the nucleus accumbens. The data show that only Δ FosB has obvious anti-depressant and pro-addictive effects after long-term expression, suggesting that $\Delta 2\Delta$ FosB cannot complement Δ FosB action.

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Screening and Identification of protein binding partners of FosB, Δ FosB, and $\Delta 2\Delta$ FosB using yeast two hybrid system

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The fosB gene, a member of the AP-1 transcription factor family, has unique properties. It is transcribed into two mature mRNAs encoding FosB and a C-terminal truncated form, Δ FosB. AP-1 complexes consist of Fos family (c-fos, fosB, fra-1, fra-2) and Jun family (c-jun, junB, junD) proteins. Jun proteins are the main component of AP-1 complexes, and activate transcription of target genes as homodimers or as heterodimer with Fos family proteins. On the other hand, Fos family proteins exhibit variable effects on AP-1 transactivity. c-Fos and FosB enhance the transactivity of Jun, while Δ FosB is less active in many cases and, in some cases, can antagonize AP-1 mediated transcription.

Our laboratory has focused on Δ FosB function in brain, and have shown that Δ FosB accumulates after prolonged exposure to stress, drugs of abuse, or other stimuli due to its unusual stability. We have shown further that Δ FosB is a key regulatory of drug and stress responses. Recently, novel binding partners of c-Fos have been reported, however, those of FosB and Δ FosB remain unexplored, except for our recent demonstration that Δ FosB is a direct binding partner for HDAC1 (histone deacetylase 1) (Renthal et al., J. Neurosci. 28:7344, 2008).

To further elucidate the functions of Δ FosB and other FosB products, we performed yeast two hybrid screening with several fragments of FosB. We identified 8 candidate binding partners with $\Delta 2\Delta$ FosB (N-terminal deletion of Δ FosB), 7 candidates with the N-terminus of FosB, and 6 candidates with the C-terminus of FosB. Binding of Δ FosB or FosB to several of these candidates has been confirmed in cell culture and in brain. One example is ATF4 (activating transcription factor 4). Together, this work promises to provide new insight into the mechanisms by which Δ FosB and other FosB gene products regulate brain function and mediate complex behavioral plasticity.

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39 **Dietary composition modulates brain mass and solubilizable A β levels in a mouse model of aggressive Alzheimer's amyloid pathology**

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Objective: Alzheimer's disease (AD) is a progressive neurodegenerative disease of the central nervous system (CNS). Recently, an increased interest in the role diet plays in the pathology of AD has 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 dietary composition modulates cerebral amyloidosis and neuronal integrity in the TgCRND8 mouse model of AD.

Methods: From 4 wks until 18 wks of age, TgCRND8 mice were maintained on one of four diets: (1) reference (regular) commercial chow; (2) high fat/low carbohydrate chow (60 kcal% fat/30 kcal% protein/10 kcal% carbohydrate); (3) high protein/low carbohydrate chow (60 kcal% protein/30 kcal% fat/10 kcal% carbohydrate); or (4) high carbohydrate/low fat chow (60 kcal% carbohydrate/30 kcal% protein/10 kcal% fat). At age 18 wks, mice were sacrificed, and brains studied for (a) wet weight; (b) solubilizable A β content by ELISA; (c) amyloid plaque burden; (d) stereologic analysis of selected hippocampal subregions.

Results: Animals receiving a high fat diet showed increased brain levels of solubilizable A β , although we detected no effect on plaque burden. Brains of mice fed a high protein/low carbohydrate diet were 5% lower in weight than brains from all other mice. In an effort to identify regions that might link loss of brain mass to cognitive function, we studied neuronal density and volume in hippocampal subregions. Neuronal density **and** volume in the hippocampal CA3 region of TgCRND8 mice tended to be lower in the mice receiving the high protein/low carbohydrate diet than in those receiving the regular chow. Neuronal density and volume were preserved in CA1 and in the dentate gyrus.

Interpretation: Dissociation of A β changes from brain mass changes raises the possibility that diet plays a role not only in modulating amyloidosis but also in modulating neuronal vulnerability. In the absence of a study of the effects of a high protein/low carbohydrate diet on nontransgenic mice, one cannot be certain how much, if any, of the loss of brain mass exhibited by high protein/low carbohydrate diet-fed TgCRND8 mice was due to an interaction between cerebral amyloidosis and diet. Given the recent evidence that certain factors favor the maintenance of cognitive function in the face of substantial structural neuropathology, we propose that there might also exist factors that sensitize neurons to some forms of neurotoxicity, including, perhaps, amyloid neurotoxicity. Identification of these factors could help reconcile the poor clinicopathological correlation between cognitive status and structural neuropathology.

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40**Regulation of opioid receptors by their endogenous ligands**

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The endogenous opioid system regulates many emotional and physiological responses mediated by the activation of mu-, delta-, and kappa-opioid receptors. The main endogenous opioid peptides identified so far result from the processing of three precursors, proopiomelanocortin, proenkephalin and prodynorphin. Although each peptide may show preference for one of the opioid receptors, they may have affinity for the others as well. By using a knockout approach, we decided to determine if absence of enkephalins and/or β -endorphin would increase levels of dynorphin-derived peptides. First, we measured the levels of Dyn A-8 and found no major changes in animals lacking enkephalins and/or β -endorphin compared to wild types. Then we measured the levels of Leu-enkephalin to determine if dynorphin was differentially processed to produce this peptide in the absence of enkephalins and/or β -endorphin in the brain. Our results showed no increase in Leu-enkephalin levels suggesting that no differential processing of dynorphin-derived peptides occurred. Since the absence of enkephalins and/or β -endorphin did not cause major effects on the levels and/or processing of dynorphin-derived peptides, we wanted to determine if there would be an effect on the activity of opioid receptors in the brain. Our results showed a decrease in mu- and delta-opioid receptor activity in the midbrain of animals lacking β -endorphin and/or enkephalins, suggesting that these peptides can regulate the activity of their target receptors. Moreover, these effects seem to be region-specific because different results were observed in the midbrain and cortex. Additional studies need to be conducted to further assess this regulation of opioid receptors.

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41**Substrate dependent effects of ethanol on growth cone responses to guidance cues**

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Ethanol toxicity in utero is a common cause of mental retardation. Careful analysis of alcohol-related neurodevelopmental disorders (ARND) over the past thirty years has served to illuminate the most common sites of brain damage and to characterize common neurological and cognitive deficits. Nevertheless, the mechanisms by which ethanol exerts its actions remain incompletely understood. The neuropathology of ARND is remarkably similar to mutations of the cell adhesion molecule, L1, which is critical for axonal outgrowth, fasciculation, migration and growth cone guidance. In the developing nervous system, axonal growth cones normally respond to a number of chemotropic and cell-contact guidance cues that act as attractants or repellants for developing projections, in order to initiate and maintain targeted growth and synapse formation. Here it is shown that at physiological concentrations, ethanol arrests motility of cortical neurons grown on poly-L-lysine (PLL), and inhibits axonal growth cone responses to semaphorin3A (sema3A), an L1-dependent guidance cue. Furthermore, growth cone responses to lysophosphatidic acid (LPA) and netrin, which function independently of L1, are also impaired in the presence of ethanol. However, when neurons are grown on an L1 substrate, neuron responses to these guidance cues are restored despite the presence of ethanol. These findings suggest that ethanol impairs growth cone function by invoking global changes in plasma membrane organization, which is required for the normal function of L1 and guidance cues.

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Excitatory presynaptic boutons contacting multiple postsynaptic specializations are termed multisynaptic boutons (MSBs). The number of MSB synapses as a percentage of all synapses increases with synaptogenic stimuli. Newly grown spines usually contact MSBs rather than single-synaptic boutons (SSBs). How MSBs might feature in synaptic development has yet to be determined. With pre- and postsynaptic double immunolabeling, we found that MSB profiles exist in cultured hippocampal neurons to a degree comparable to that reported for MSB synapses *in vivo*. In our cultures, the number of immunolabeled MSB profiles as a percentage of all synaptic profiles remained relatively constant over time. To further characterize MSBs and their contacting spines in culture, we obtained spine pair parameters that could be applied to identify MSB-contacting spines in fluorescence-filled neurons. We imaged GFP-filled cultured neurons by laser-scanning confocal microscopy (LSCM) then used correlative light and electron microscopy (CLEM) to verify MSB-contacting spine pairs. We evaluated four criteria for MSB spine pairs compared to control pairs: spine head distance, spine orientation, spine head volume, and spine type. Compared to control spine pairs, MSB-contacting spine pairs were approximately 30% closer to each other, more frequently angled towards each other, had more similar head volumes, and were more likely to be mushroom spines. The similarity in spine volume and type for MSB spine pairs is consistent with the notion that synaptic stimulation is a primary influence on spine morphology. To evaluate for the first time how MSBs interact with their contacting spines in live neurons, we used our spatial and morphological criteria to identify MSB-contacting spine pairs in GFP-filled neurons in live LSCM-imaged cultures. The GFP-filled neurons were co-cultured with neurons containing DsRed:VAMP-labeled boutons. In live images taken hourly over at least 15 hours we identified examples of MSBs with both persistently and transiently contacting spines.

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Brain amyloid- β oligomer level – not plaque burden – correlates with memory deficit in a new mouse model of Alzheimer’s disease

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Objectives: Recent evidence suggests that high molecular weight soluble oligomeric A β (oA β) assemblies (also known as A β -derived diffusible ligands, or ADDLs) may represent a primary neurotoxic basis for cognitive failure in AD. To date, in vivo studies of oA β /ADDLs have involved injection of assemblies purified from the cerebrospinal fluid (CSF) of human subjects with Alzheimer’s disease or from the conditioned media of A β -secreting cells into experimental animals. We sought to study the bioactivities of endogenously formed oA β /ADDLs generated in situ from the physiological processing of human APP transgenes.

Methods: We produced and histologically characterized single transgenic mice overexpressing APP^{E693Q} or APP^{E693Q} X PS1 Δ E9 bigenic mice. APP^{E693Q} mice were studied in the Morris water maze (MWM) task at 6 and 12 months of age. Following the second MWM evaluation, mice were sacrificed, and brains were assayed for A β total, A β 40, A β 42, and oA β /ADDL by ELISA and were also histologically examined. Based on results from the oA β /ADDL ELISA, we assigned individual APP^{E693Q} mice to either an “undetectable oA β /ADDLs group” or a “readily detectable oA β /ADDLs group”. A days-to-criterion (DTC) analysis was used to determine delays in acquisition of the MWM task.

Results: Both single transgenic and bigenic mice developed intraneuronal accumulation of APP/A β , though only Dutch APP^{E693Q} X PS1 Δ E9 bigenic mice developed amyloid plaques. The APP^{E693Q} mice did not develop amyloid plaques at any age studied, up to 30 months. APP^{E693Q} mice were tested for spatial learning and memory, and only 12-month old APP^{E693Q} mice with readily detectable oA β /ADDLs displayed a significant delay in acquisition of the MWM task when compared to NTg littermates (p=0.01).

Interpretation. Recently, several groups have reported early deficits in acquisition of spatial learning and memory tasks among patients with hippocampal-related mild cognitive impairment or AD, and among animal models of AD, which occur prior to deposition of amyloid plaques. We are the first to demonstrate that cerebral oA β /ADDL assemblies generated in brain in situ from human APP transgenes are associated with cognitive impairment in a dose-dependent manner. We propose that a DTC analysis may be a sensitive method for assessing the cognitive impact in mice of endogenously generated oligomeric human A β assemblies.

NOTE: This work was recently published (Gandy et al, *Annals of Neurology*, April 2010, 10.1002/ana.22052).

SPONSORSHIP & DISCLOSURE

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Mechanisms of memory enhancement via hippocampal insulin-like growth factor II

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There is currently little known about mechanisms of memory enhancement. Memory strengthening depends on consolidation, the process by which newly learned information becomes long-lasting as it goes from a labile state into one resistant to disruption. We have recently shown that the insulin like growth factor II (IGF-II), a growth factor kinase belonging to the superfamily of IGF's, significantly enhances inhibitory avoidance (IA) memory and prevents forgetting when administered into the hippocampus immediately after training or retrieval. However, in order to be effective, IGF-II must be administered within a sensitive period from training; IGF-II injections enhance reconsolidation when injected after a retrieval session 1 day post-training, but not after a retrieval session 14 days post-training. IGF-II mRNA and protein is significantly upregulated in the hippocampus following IA training, and the upregulation requires the transcription factor C/EBPbeta. Post-training hippocampal injections of IGF-II, but not IGF-I, persistently enhance memory in a dose-dependant manner. Furthermore, the enhancement requires de novo protein synthesis and IGF-II receptors, but not IGF-I receptors. Here we asked the question of whether the effect of IGF-II on memory enhancement is general, and acts on different brain regions and learning tasks. We found that despite the fact that IA is a task that is dependant on both the hippocampus and amygdala, IGF-II does not enhance IA memory when injected into the amygdala following training. On the other hand, hippocampal injections of IGF-II enhance contextual fear conditioning and not cue fear conditioning memory. These data suggest that IGF-II may play a role in enhancing hippocampal-dependent but not amygdala-dependent representations/mechanisms. . We are now examining the effect of IGF-II injected into hippocampus, amygdala or prefrontal cortex on IA, fear conditioning and spatial learning tasks. Further work will elucidate whether IGF-II affect hippocampal but not amygdala mechanisms.

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45 The astrocyte-neuron lactate-shuttle is essential for hippocampal long-term memory formation

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The cross-talk between astrocytes and neurons in learning, memory and cognitive functions is poorly understood. Astrocytes provide metabolic support to neurons. Glycogen, which is primarily stored in astrocytes, contributes to neuronal function by being broken down into lactate. Previous studies have shown that inhibition of glycogenolysis disrupts the retention of an aversive memory in chick, and this disruption was rescued by L-Lactate, suggesting that the metabolic role of astrocytes is important for memory consolidation (Gibbs et al 2008). Here, we investigated the role of glycogenolysis and lactate transport in the consolidation of a hippocampal-dependent memory in rat. We found that blocking glycogenolysis with the glycogen phosphorylase inhibitor, 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) bilaterally into the dorsal hippocampus before or after inhibitory avoidance (IA) training significantly blocked long-term memory without affecting short-term memory. In agreement, DAB also blocked in vivo-induced CA1 long-term potentiation (LTP). Furthermore, blocking the lactate transport from astrocytes to neurons with antisense-mediated disruption of the MCT1 and MCT2 completely and persistently blocked memory consolidation without affecting short-term memory. Importantly, exogenous administration of L-lactate rescued the memory and LTP impairments produced by DAB. L-lactate but not glucose rescued the memory impairment produced by MCT1 disruption, but did not affect the amnesia produced by MCT2 knock-down. Finally, molecular changes known to underlie long-term plasticity and memory formation including induction of activity-regulated cytoskeletal-associated protein (Arc), phosphorylated cAMP response element binding protein (pCREB) and phosphorylated cofilin (pcofilin), were completely blocked by DAB and significantly rescued by L-lactate. These findings are in agreement with the astrocyte-neuron lactate shuttle hypothesis (ANLSH), which proposes that the lactate released from astrocytes is transported into neurons and used for neuronal functions including fuel activity-dependent energy demands (Magistretti 2006). We conclude that astrocytic glycogenolysis and lactate shuttle play an essential role in the hippocampus during long-term memory formation.

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Comparison of neuronal morphology in pyramidal neurons of the CA1, dentate gyrus, and prefrontal cortex of APP knockout and APLP2 knockout mice

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Abstract:

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. Many of these functions extend to the two homologues of APP, APLP1 and APLP2. Studies have shown synaptic deficits and decrease in spines prior to the accumulation of plaques in AD and in APP transgenic mouse models, which have been attributed to A β -induced toxicity. Furthermore, APP^{-/-} mice have been shown to have cognitive and motor function deficits (at 4 and 10 months). However, whether synapse numbers are altered in APP^{-/-} is controversial. In this study, we examined the possible changes in neuronal morphology, including dendritic spine structure in APP^{-/-} mice to determine whether APP has an effect on dendritic integrity. We also compared the possible changes in neuronal morphology, in aged APP^{-/-} mice to aged APLP2^{-/-} mice to determine if any alterations seen were due to lack of APP. 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 previously specified regions. Neurons were traced using NeuroLucida (MBF Bioscience) software and Sholl analysis was performed to quantify dendritic length and complexity. We found that 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. There were no significant differences in apical or basal dendritic length or complexity in the same regions of young mice. 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 alone, without the two orthologs APLP1 and APLP2, 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.

47 The pathological study of PD related G2019S LRRK2 mutation in BAC transgenic mice brain

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The leucine-rich repeat kinase 2 (LRRK2) G2019S mutation is recognized as the leading cause of familial and some sporadic forms of Parkinson disease (PD). Although the abnormality of LRRK2-G2019S kinase activity is suggested highly related to the pathogenesis of PD, the pathological function of LRRK2 remains unknown. Using BAC (bacterial artificial chromosome) technique, our lab developed FLAG-tagged wild-type LRRK2 (LRRK2-Wt) transgenic mice and G2019S mutant mice (LRRK2-G2019S). The transgenic mice overexpress LRRK2-Wt or LRRK2-G2019S at the similar levels (approximately 6-8 folds over the endogenous). Although previous study indicated a significant increase in the brain of LRRK2-G2019S kinase activity over that of LRRK2-Wt, the nigrostriatal dopaminergic pathway observed with TH staining did not display any obvious terminal degeneration in striatum or neuronal loss in substantia nigra pars compacta (SNpc) up to 18 months old. However, we found that dopamine transmission and striatal dopamine content is significantly reduced in LRRK2-G2019S mice. In contrast, LRRK2-Wt mice have elevated dopamine release, accompanied with enhanced motor function. Recently we found in cerebral cortex as well as cerebellar Purkinje cell layer of LRRK2-G2019S mice that there is dramatic accumulation of LRRK2-positive staining in the glial cell, which is missing in both LRRK2-Wt and non-transgenic mice brain. Glial fibrillary acidic protein (GFAP) staining demonstrated increased astrocyte activation in frontal cortex layer of LRRK2-G2019S mice over that of non-transgenic control, whereas over-expression LRRK2-Wt mice shows effective protection to astrocyte gliosis. Overall, these results imply a possible role of gliosis in the pathogenesis of PD-related LRRK2-G2019S mutation. This project is funded by M. J. Fox Foundation for Parkinson's Research and NIH/NINDS.

The role of Wnt-beta-catenin signaling in a mouse model of depression

Matthew B. Wilkinson, Jane Magida, Caroline Dias, Pamela J. Kennedy, Michelle Mazei-Robison, Herb Covington, and Eric J Nestler

Major depressive disorder (MDD) is one of the most common mental illnesses, but is still poorly understood, especially with regard to the disconnect between treatment initiation and the delayed clinical efficacy of antidepressants. Our laboratory has 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. We used ChIP-chip assays--chromatin immunoprecipitation followed by genome wide promoter array analyses--to study the effects of chronic defeat stress on chromatin regulation in the 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 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. The strong correlation in regulated genes between animals treated with imipramine and those naturally resistant to the effects of the stress paradigm provide us with a valuable set of data that we are now using to identify novel genes that may play a role in MDD and its treatment.

Ingenuity pathway analysis was utilized to identify families of genes involved in the depression-like phenotype in these animals. We found that genes related to inflammation (e.g. NF-kappaB, interleukins), gene transcription (e.g. Spi-C), and actin remodeling were three major classes that warrant further study. We are now focusing on validation of these target genes at the transcript and protein levels, and will then use viral vectors to examine directly their role in the social defeat model. We also plan to use ChIP analyses with other stress-activated transcription factors (e.g., delta-FosB) and histone marks to more fully understand the regulation of these genes. Additionally, ChIP followed by Solexa sequencing will allow us to determine the exact binding sites of these transcription factors. These data allow us to more precisely define the genes and pathways that play a role in MDD in brain reward pathways.

49 Epigenetic modification in human and rat tissue in association with prenatal cannabis exposure

Claudia V Morris, Henrietta Szutorisz, Jennifer Dinieri, and Yasmin L Hurd

Approximately 4% of women in the United States report using illegal drugs during pregnancy, with 75% admitting marijuana use. Prenatal cannabis exposure has been linked to increased risk of developing neuropsychiatric disorders later in life, including drug addiction. The major psychoactive component of marijuana is Δ^9 -tetrahydrocannabinol (THC) and the neuroanatomical pathway most vulnerable to THC effects is the mesolimbic reward circuitry. Our lab has previously shown that prenatal cannabis exposure is associated with changes in components of the mesolimbic pathway in both humans and rats, leading to altered protein and gene expression levels that persist into adulthood. Of these changes, the long-term reduction of dopamine receptor D2 (Drd2) gene expression in the ventral striatum appears to be the most significant consequence of prenatal marijuana use. The precise regulatory mechanisms behind these enduring effects have yet to be defined but epigenetic modification is an intriguing candidate, especially as a number of studies have demonstrated that psychoactive drugs can induce epigenetic alterations. Epigenetics refers to changes in gene expression due to modalities independent of changes to the genetic code, such as histone modifications that have permissive or repressive effects on gene transcription. The aim of our studies is to determine whether changes in gene expression in association with prenatal cannabis exposure are due to gene-specific epigenetic modification or global alterations in chromatin structure within the striatal reward circuitry. Preliminary analysis of histone H3 lysine 4 trimethylation by chromatin immunoprecipitation has indicated a decrease of this permissive mark at both the dopamine receptor 1 (Drd1) and Drd2 genes in the nucleus accumbens of adult rats with prenatal THC exposure. To address the specificity of such changes, the ventral striatum, dorsal striatum, and prefrontal cortex will be dissected from marijuana-exposed human fetal brain tissue and rats exposed to THC during gestation. Histone extracts from these regions will be electrophoresed and western blots will be performed to compare the levels of various histone modifications between cannabis-exposed and control subjects. Funded by NIH grants DA120320 & 1T32MH087004-01A1.

50 Phenoxy herbicides and fibrates potently inhibit the human T1R3 chemosensory receptor.

Emeline L. Maillet, Robert F. Margolskee and Bedrich Mosinger.

The sweet-sensing receptor is a hetero-dimer of two class-C G-protein-coupled receptors, T1R2 and T1R3. T1R3 receptor also dimerizes with T1R1 and forms a major receptor for amino acids conveying umami taste. Recently, T1Rs receptors, which were thought to be specific for taste cells located in papillae at the surface of the tongue and soft palate, were found to be expressed outside of the taste system, in chemosensory and endocrine cells in gastrointestinal tract, kidney, liver, testis, exocrine pancreas and lymphocytes. Activated sweet receptors in taste cells signal the presence of carbohydrate-rich foods to the brain; the same receptors in intestinal enteroendocrine cells regulate secretion of glucagon-like peptide-1 (GLP-1) and induce expression of sodium-glucose co-transporter-1 (SGLT1) leading to enhanced absorption of carbohydrates. These studies indicate that T1Rs receptors may have roles in glucose homeostasis and energy metabolism and that their altered activity may contribute to pathologies such as type II diabetes and obesity.

We examined effects of compounds chemically related to the sweet receptor inhibitor lactisole, including widely used phenoxy-auxin herbicides and anti-lipidemia fibrate drugs. We have determined that widely used herbicides 2,4DP, 2,4D and MCPP potently inhibit the human T1R2/3 receptor responses to sweeteners. We also found that clofibrate and related anti-lipid drugs such as bezafibrate and fenofibrate, also inhibit the human T1R2/3 receptor. We determined that these compounds act specifically on the human form of the T1R3 and not on the rodent form. They act as NAM (negative allosteric modulator) of the transmembrane portion (TMs) of human T1R3.

Fibrates' biological activity had been thought to occur mainly through activation of PPAR α . Our results show that fibrates and phenoxy-herbicides inhibit human T1R3 with potency comparable to that shown by fibrates acting on PPAR α . T1R3 thus may be a primary target of fibrates in human, underlying certain of their biological effects in treating hyperlipidemia and type II diabetes. Likewise, phenoxy-herbicides' effects on T1R3 may underlie certain long-term side effects in humans that due to the species differences in T1R3 would have gone undetected in toxicological animal studies.

Epigenetic regulation of 5HT2A-mGlu2 receptor complex expression by chronic atypical antipsychotics.

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The serotonin and glutamate systems are suspected in the etiology and pathophysiology of schizophrenia, as well as in the mechanism of action of antipsychotic drugs. We have recently demonstrated that the serotonin 5-HT_{2A} receptor (5HT2A) and the metabotropic glutamate receptor 2 (mGlu2) interact to form a functional receptor complex in brain and tissue culture preparations. Accumulating evidence suggests that the 5HT2A-mGlu2 receptor complex may be responsible for the disordered thought processes in schizophrenia brain. In schizophrenia patients, usually two to three weeks or more are required to demonstrate obvious beneficial effects of antipsychotics. Maximum benefits in chronically ill patients may require several months. These observations have suggested the involvement of altered gene expression in antipsychotic action. Interestingly, our chromatin immunoprecipitation (ChIP) data demonstrate that chronic, but not sub-chronic, treatment with clozapine elicits histone modifications at the promoter regions of 5HT2A and mGlu2 that correlate with their level of expression in mouse frontal cortex. No histone modifications were detected at 5HT2C, mGlu3 or dopamine D2 promoters. Recent preclinical and clinical studies suggest that drugs such as valproate (VPA), one of whose functions is to act as a nonspecific histone deacetylase (HDAC) inhibitor, are efficacious in the treatment of schizophrenia when given systemically in combination with atypical antipsychotics. Our in vitro transcriptional assay data demonstrate that HDAC inhibitors, such as trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA), and MS-275, increased the transcriptional activity of mGlu2. Notably, HDAC2, and not HDAC1, was bound to the promoter region of mGlu2, and not 5HT2A, in mouse frontal cortex. Based on these results, we propose that the antipsychotic effects of HDAC inhibitors in combination with atypical neuroleptics may be due, at least in part, to the epigenetic regulation of 5HT2A-mGlu2 receptor complex expression in frontal cortex.

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2010 UPCOMING EVENTS

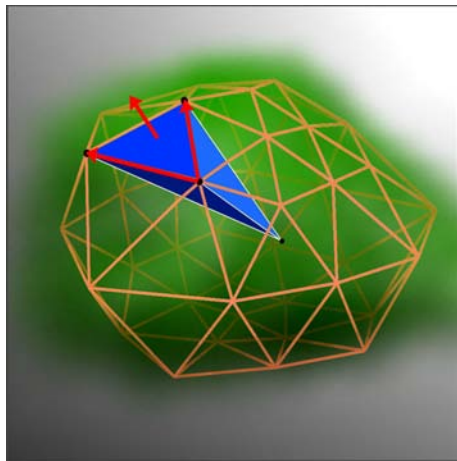


photo by the Wearne and Hof Labs

July

August

Grad School Classes Begin
August 23, 2010

September

October

MD/PhD Retreat
October 8th and 9th, 2010

November

December

Society for Neuroscience
Nov. 13-17, in San Diego, Calif.

Grad School Winter Party
December 17, 2010

Graduate Program information

Last year at this Retreat, we introduced a number of significant changes to the Neuroscience MTA, ranging from new advanced elective courses, to new Core course leadership, to a streamlined format for the qualifying examination. We are pleased to report that these changes have been largely a major success, the best testament to which has been the positive feedback from the Neuroscience students. In the works for the upcoming year is a Clinical Neuroscience advanced elective course, co-directed by Jenny Zou and Eric Nestler and team-taught by a diverse set of clinical research faculty and physicians. This course will likely be taught in the spring of every other year, so watch the course schedules carefully. In the meantime, we want to hear from you about what works, what doesn't work, and as always, we remain open to suggestions for improvement.

We are also pleased to report on the many successful NRSA, NSF and other agency grants that our current graduate students garnered during this past year. Keep up the great work! It is a significant and prestigious achievement, particularly in this competitive funding climate. It should certainly be a goal of every eligible student to apply for predoctoral grants or fellowships (take note, Mentors!). Finally, we look forward to welcoming a terrific pool of new Graduate Students matriculating this fall. The talent, diversity, and breadth of our students only continues to rise.

George Huntley
and Stephen Salton

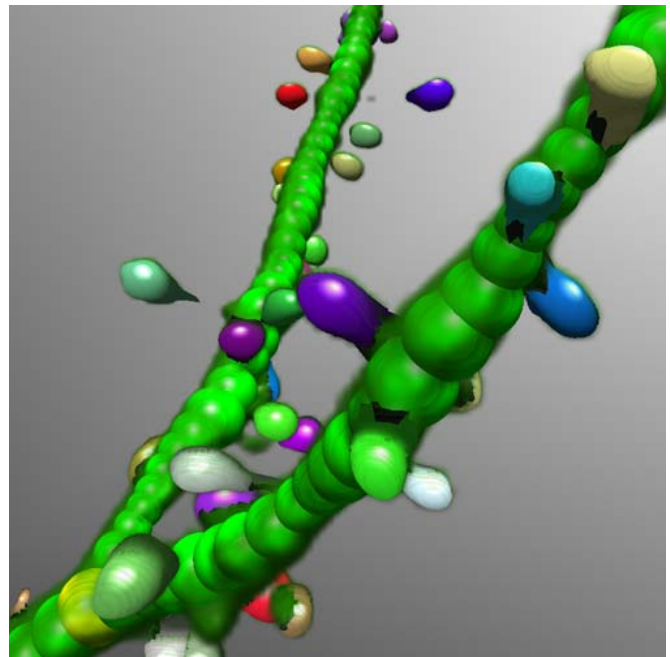


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