Alcohol tolerance:
The view from BK
...and the Institute of Neurobiology
San Juan,
Tolerance is a many-splended thing

Why do we do what we don’t want to do?
Molecules and addiction
What is Tolerance?

- Progressive adaptation
- Tolerance contributes to dependency and increased consumption.
- Acute tolerance is a predictor of the likelihood to develop dependence to alcohol.

Adaptation is absolutely necessary for life to exist!

BK Channels: molecular tolerance

- Calcium and voltage activated K+ channel
- Tetrameric
- Single α gene = multiple splice variants
- Auxiliary β1-β4 subunits

Orio et al., 2002
“...Verily, Alcohol shall know
Protein not only by her
structure, but as well, by the
company that she keeps...”

Molecular adaptation: Potential mechanisms

- Lipid environment
- α subunit splicing
- Post-translational
- Breeding pressure
- Exercise
- Climate change

Presented at the 13th RCMI International Symposium on Health Disparities | December 9-13, 2012 | San Juan, Puerto Rico
Molecular tolerance

Channels open
Channels closed

24 hr EIOH

Molecular Alcohol Tolerance

Cell body miRNA
and Splicing decisions

Pietrzykowski et al. Neuron, 2008
A microRNA (abbr. miRNA) is a small RNA molecule (ca. 22 nucleotides) that functions in the post-transcriptional regulation of gene expression. Encoded by eukaryotic nuclear DNA, miRNAs function via base-pairing with complementary sequences within mRNA molecules, usually resulting in gene silencing via translational repression or target degradation. The human genome may encode over 1000 miRNAs, which may target about 60% of mammalian genes.
miR-9 mRNA is present in SON neurons and EtOH downregulates its expression.

Consequences of the isoform switch:

- p27
- Insertless
- STREX

Before EtOH: p27, Insertless, STREX; After EtOH: Insertless, STREX

Decreasing sensitivity to alcohol insensitive.

Neuronal cell body responds to the presence of alcohol by a mechanism simultaneously down-regulating BK channel gene expression and switching splice variants from alcohol-sensitive to alcohol insensitive.
miR-9 may integrate multiple targets

A model of miRNA-regulated neuronal adaptation to EtOH

Stress

Acquired alcohol insensitivity

Figure 8
Additional miR-9 targets
What are the dynamics of miR-9 upregulation and decline, ...and what is the subsequent time course of BK translation and membrane insertion into the neuronal membrane?

Alcohol stimulates release of dopamine in the nucleus accumbens. Extensively studied for its important role in reward and the reinforcing effects of drugs of abuse.
Are there temporal and concentration dependent switches tripped by drug exposure,?

Can tolerance be described by a "trigger function", in which continued presence of the drug is unnecessary once the machinery of tolerance is initiated?

Can molecular-cellular models explain behavioral tolerance studies that suggest such switches do exist.

Is there a relationship between EtOH exposure protocol (pattern) and the character of tolerance that develops in striatal neurons?
Approach

- Develop a P8 striatal culture in order to temporally administer and withdraw EtOH with precision.
- Pre-expose cultured rat P8 striatal neurons to 20 mM ethanol (legal intoxication) for 1, 3, or 6 hours followed by various withdrawal periods.
- Assess alcohol sensitivity (inside-out patch clamp) at various withdrawal time points.

Rationale – 1 hr vs. 6 hr Exposure Protocol

- Ettenberg et al. (1998) One hour, but not six hours, of daily access to self-administered cocaine results in elevated levels of the dopamine transporter.
- Dohrman et al. (2002) 6 hr of EtOH translocated the catalytic subunit of PKA from the golgi area to the nucleus.

Etokh exposure time controls persistence of tolerance: 3 vs. 6 hrs
Striatal neurons obtained from mice 24 hrs after in vivo alcohol exposure

Some potential mechanisms for tolerance "switch"
- Epigenetic mechanisms (DNA methylation and chromatin reorganization)
- Post-translational modifications (phosphorylation, glycosylation)
- Post-transcriptional changes (splicing)
- Transcriptional alterations (changes in subunit composition)

Second Question
Does a 6 hr EtOH exposure induce changes in BK channel composition that are not present with a 3 hr exposure?
Gating properties of BK channels differ during withdrawal after a 6 hr but not a 3 hr exposure to 20 mM EtOH.

Mean open (MOT) and closed times (MCT) of BK channels are different during withdrawal after a 6 hr but not a 3 hr exposure to 20 mM EtOH.

Is it Strex?

6 but not 3 hours induces an upregulation of STREX within 24 hr of withdrawal.
HEK expression of BK strex looks just like the post 6-hr BK!

Indeed, there is a "switch" triggered during a 6 hr EtOH exposure that is not triggered during a 3 hr exposure.

Continued exposure to EtOH is not necessary for the maintenance of tolerance after a 6 hr exposure.

Implications for consequences of drinking patterns in people.

Conclusions

1. Indeed, there is a "switch" triggered during a 6 hr EtOH exposure that is not triggered during a 3 hr exposure.

2. Continued exposure to EtOH is not necessary for the maintenance of tolerance after a 6 hr exposure.

3. Implications for consequences of drinking patterns in people.

Modulation of EtOH sensitivity by α splice site variation (HEK transfection).

### Table: Activity Assay

<table>
<thead>
<tr>
<th>Treatment</th>
<th>p27</th>
<th>Insensitive</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.95 ± 0.16</td>
<td>6.53 ± 0.80</td>
<td>7.78 ± 0.75</td>
</tr>
<tr>
<td>SE1-24 h</td>
<td>1.24 ± 0.34</td>
<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
</tr>
<tr>
<td>SE2-24 h</td>
<td>1.24 ± 0.34</td>
<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
</tr>
<tr>
<td>SE3-24 h</td>
<td>1.24 ± 0.34</td>
<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
</tr>
<tr>
<td>SE4-24 h</td>
<td>1.24 ± 0.34</td>
<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
</tr>
<tr>
<td>SE5-24 h</td>
<td>1.24 ± 0.34</td>
<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
</tr>
<tr>
<td>SE6-24 h</td>
<td>1.24 ± 0.34</td>
<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
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<tr>
<td>SE7-24 h</td>
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<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
</tr>
<tr>
<td>SE8-24 h</td>
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<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
</tr>
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<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
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<tr>
<td>SE10-24 h</td>
<td>1.24 ± 0.34</td>
<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
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<tr>
<td>SE11-24 h</td>
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<td>6.10 ± 0.62</td>
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<tr>
<td>SE12-24 h</td>
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<td>6.10 ± 0.62</td>
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<tr>
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<td>7.85 ± 0.71</td>
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<tr>
<td>SE14-24 h</td>
<td>1.24 ± 0.34</td>
<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
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<tr>
<td>SE15-24 h</td>
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<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
</tr>
<tr>
<td>SE16-24 h</td>
<td>1.24 ± 0.34</td>
<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
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<tr>
<td>SE17-24 h</td>
<td>1.24 ± 0.34</td>
<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
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<tr>
<td>SE18-24 h</td>
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<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
</tr>
<tr>
<td>SE19-24 h</td>
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<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
</tr>
<tr>
<td>SE20-24 h</td>
<td>1.24 ± 0.34</td>
<td>6.10 ± 0.62</td>
<td>7.85 ± 0.71</td>
</tr>
</tbody>
</table>

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Tian et al. 2000
Pietrzykowski et al. (Neuron)
Persistence of tolerance depends on length of alcohol exposure:
- 6 hr exposure: persistent suppression of EtOH sensitivity for 24 hrs
- 3 hrs: resensitized to the drug after a few hours
- Changes in isoform composition may contribute to this persistent insensitivity.

Decrease in current density after 6 hr EtOH exposure is blocked by Cyclohexamide (i.e. protein synthesis dependent)
Molecular tolerance depends upon protein synthesis

Table 1. List of Proteins of Interest

Clik-it

The holy grail: Molecules to behavior 

PNAS, 2009
Beta subunit controls acute tolerance and drinking behavior

Playing with lipids

Court of public opinion: Question: Where does ethanol act?
Experimental

1. Patch recording

2. Planar bilayer recording

Transfect and express Hslo mRNA

Molecular:
- Shape (packing)
- Charge
- Chain length
- Saturation
- Rigidity
- Domains
- Bilayer thickness

Cholesterol affects EtOH action

Graph showing NPo Ethanol Presented at the 13th RCMI International Symposium on Health Disparities | December 9-13, 2012 | San Juan, Puerto Rico

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Schematic Representation of Stacked Membrane Bilayers as an X-ray Diffraction Lattice

How does cholesterol do it?

Cholesterol and its enantiomer, Ent-cholesterol.

Ent-Cholesterol is mirror image of Nat-cholesterol, and can be distinguished only by plane-polarized light or by interaction with another chiral molecule.
Contributors to work discussed today:
- Alex Dopico
- Andrzej Pietrzykowski
- Gilles Martin
- Patricia Wynne
- Cristina Velazquez
- Loyda Melendez
- Juliana Perez
- Chunbo Yuan
- Alexandra Bernardo
- John Crowley
- Robert O'Connell
- Jose Garcia
- Ryan Friesen
- José Lemos

Ethanol sensitivity and rapid alcohol tolerance of BK$_{	ext{Ca}}$ channels in HEK293 cell and lipid bilayers of different thicknesses

Guiding principle: Direct interaction of EtOH with channel protein...
However, lipid environment has significant influence.

How to change the thickness of lipid bilayers

PC    SPM
Summary

• Acute tolerance: observed in HEK 293 cell patches, and bilayer system.
• Precludes the necessity for transcriptional and translational involvement.
• Intrinsic to the protein.
Kinase action (and influence on tolerance) is beta subunit dependent
Show protein synthesis slides

Figure 5. BK channel can be recorded from embryonic rat hippocampal primary cultures. Voltage and calcium dependence of BK channels in embryonic rat hippocampal cultures. A, Representative image of pyramidal neurons 2 weeks in culture. Staining DAPI = blue (nucleus), MAP2 = green (dendrites), and Synapsin I = red (presynaptic marker). B, Representative single channel traces of inside-out patches at different potentials in the presence of 10 μM free [Ca2+]. C and O, adjacent to the traces, indicate the closed and the open states, respectively. C, Unitary conductance was determined by fitting the I–V relationship by linear regression (n = 5) ± S.EM. D, Representative single channel traces at increasing concentrations of calcium. RACHAEL KEAOUHIKANEALEI GONZALES

University of Hawaii at Manoa
Altered Brain Microstructure is Associated with Higher Cortisol Levels in Chronic Marijuana Users

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John A. Burns School of Medicine
University of Hawaii
13th RCMI International Symposium on Health Disparities
December 11, 2012
San Juan, Puerto Rico

In Hawai‘i, 22.2% of admissions to drug rehabilitation clinics were for marijuana as the primary drug of abuse, second only to methamphetamine.

In 2009, four million U.S. citizens aged 12 or older used MJ daily.

(1) United Nations Office on Drugs and Crime, 2010; (2) SAMHSA, 2008-2009 NSDUHs; (3) US DHHS, 2005

Marijuana & the Brain

• There are a limited number of studies evaluating in brain structure in adult chronic marijuana users.

• The results of these studies conflict as to whether marijuana use is associated with structural alterations.

**Reasons for Inconsistency**

- Abstinent versus active use\(^1\)\(^-\)\(^5\)
  - After 28 days of abstinence, neurocognitive function is no longer impaired\(^6\)
- Comorbid drug use\(^2\)\(^,\)\(^4\)
  - Excess alcohol is associated with impaired myelination and white matter development\(^3\)


**Cannabinoid (CB1) Receptors**

- Main endogenous targets of THC\(^1\)
- Expressed within brain white matter and by oligodendrocytes\(^2\)
- Shown to influence brain white matter development\(^3\)
  - More than half of long-term, MJ-using adults report to have started smoking before the age of 18\(^4\).

(1) Freund et al., 2003; (2) Molina-Holgado et al., 2002; (3) Ashtari et al., 2007; (4) SAMHSA, 2005

**Diffusion Tensor Imaging**

- 3 Tesla MRI Scanner
- Diffusion Tensor Imaging (DTI)
  - DTI measures the motion of water molecules to detect brain microstructure\(^1\)\(^-\)\(^2\)

(1) Alcata et al., 2009; (2) Jang et al., 2009
THC & Cortisol

• Acute THC can increase cortisol levels\(^1\) and increased levels of cortisol may be neurotoxic\(^2,3\).
• While, chronic THC administration:
  • (1) down-regulates CB1 receptors\(^4\)
  • (2) suppresses oligodendrocyte ability to produce and maintain white matter\(^4,5\)

1. Ranganatha et al., 2009
2. Swaab et al., 1994
3. Frodl et al., 2012
4. Romero et al., 2007
5. Grigorenko et al., 2002

Hypotheses

• We hypothesize that active chronic adult marijuana users will have microstructural alterations in brain white matter possibly mediated by cortisol levels.
• Additionally, we hypothesize that changes in microstructure will correlate with cortisol levels.

Methods

• All Participants:
  • Men or women, 18-45 years of age
  • Not dependent on any illicit drug or alcohol
  • No history of chronic medical, psychiatric, or neurological illnesses
• Marijuana Users:
  • Marijuana use 6-7 days/week for at least one year
  • Positive urine toxicology for THC
Large Deformation Diffeomorphic Metric Mapping (LDDMM)

- Automated transformation matrix that maps your patient’s brain onto an atlas

Standard Atlas

Marijuana Use Characteristics

<table>
<thead>
<tr>
<th>Marijuana Users (n = 27)</th>
<th>Male (n=15)</th>
<th>Female (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age of first use (yrs)</td>
<td>15.5 ± 0.8</td>
<td>16.3 ± 0.5</td>
</tr>
<tr>
<td>Mean daily use (g/day)</td>
<td>4.8 ± 1.9</td>
<td>2.8 ± 0.8</td>
</tr>
<tr>
<td>Mean duration use (yrs)</td>
<td>10.1 ± 2.3</td>
<td>6.9 ± 1.6</td>
</tr>
<tr>
<td>Lifetime exposure (g)</td>
<td>16,683 ± 7360</td>
<td>4947 ± 1197</td>
</tr>
</tbody>
</table>

No significant differences
Young adult chronic and active marijuana users, showed microstructural abnormalities, with lower diffusion in three white matter (WM) regions as hypothesized.

Discussion

- Young adult chronic and active marijuana users, showed microstructural abnormalities, with lower diffusion in three white matter (WM) regions as hypothesized.

Superior Longitudinal Fasciculi
Middle Temporal WM
Posterior Corona Radiata
Discussion

- Lower diffusivities suggests more compact axonal fibers, possibly resulting from altered brain development.
  - Chronic and early MJ use may adversely impact maturation processes such as synaptic pruning\(^1\) and white matter development\(^2\).

\(^{1}\) Herkenham et al., 1992, \(^{2}\) Molina-Holgado et al., 2002, \(^{3}\) Jia et al., 2009, \(^{4}\) Grigorenko et al., 2002

Discussion

- The correlation between the MJ users' cortisol levels and radial diffusivity, which is putative of myelin loss\(^1\), suggests that higher levels of cortisol in the brain for prolonged periods of time may be neurotoxic to brain white matter microstructure.

\(^{1}\) Song et al., 2002

Limitations

- Accuracy of self-reported drug history
  - Urine toxicology performed to verify recent use
  - Improve with timeline-follow back interviews\(^1\) and hair analyses

\(^{1}\) Chang et al., 2006
Take Home Message

- Chronic and active marijuana use in adults is associated with disrupted white matter integrity which may be related to higher cortisol levels.

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"RCMI Multidisciplinary And Translational Research Infrastructure Expansion, (RMATRIX)"

NIMHD
National Institute on Minority Health and Health Disparities

Mahalo nui loa!

- Dr. George King
- Dr. Rosanne Harrigan
- Dr. Linda Chang
- Dr. Thomas Ernst
- The Neuroscience & MRI Research Team

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ISRI (International Society for Research on Impulsivity): impulsivity is defined as 
"behavior without adequate thought, the tendency to act with less forethought, or 
a predisposition toward rapid, unplanned reactions to internal or external stimuli without 
regard to the negative consequences of these reactions."

Impulsivity is defined as the tendency to act with less forethought, or a predisposition toward rapid, unplanned reactions to internal or external stimuli without regard to the negative consequences of these reactions.
**Impulsivity**

- Associated with:
  - Substance use disorders
  - Addiction
  - Neurodevelopmental disorders (e.g., attention deficit hyperactivity disorder, autism, schizophrenia, etc.)
- Heterogeneous etiology
- Mechanisms – largely unknown

**Addiction**

- No cure
- More effective interventions needed
- Animal models: primates, rodents
- Simpler model system likely beneficial
- Genetic & environmental factors
  - Dopamine: linked to addiction and impulsivity
    - e.g., Parkinson’s patients – impulse behaviors
- Hypothesis: dopamine is central for crucial for impulse control and impulsivity caused by dysregulated dopamine underlies addiction

**Why flies?**

- Advanced genetics & genome database
- Fewer functional redundancies
- Remarkable functional conservation
- Short life cycle
- Genetic homogeneity
- Diverse behavioral plasticity
- Relatively simple nervous system
Dopamine

- a major monoamine neuromodulator in all “animals”
- attention, emotion, motivation, cognition, reward, motor control, learning & memory, etc.
- ADHD, schizophrenia, autism, drug addiction, Parkinson’s disease, etc.

Dopamine receptors – GPCR
- dDA1 – increase cAMP – D1
- DAMB – increase cAMP & Ca²⁺ – D5
- dD2R – inhibit increase in cAMP – D2, D3, D4

Dopamine transporter (DAT)
- reuptake extracellular DA
- modify neural activities

Dopamine mediates behavioral disinhibition
-- motor and cognitive impulsivity--
in Drosophila

Anti-TH IR in the fly brain (dopamine neurons)
The male fly normally courts the female fly. It has the specialized brain circuit to inhibit courtship toward other mature male Drosophila. Under the influence of alcohol, male flies actively court other males - behavioral disinhibition. More courtship with recurring experiences - behavioral sensitization. 

Ethanol-induced behavioral disinhibition

Dopamine neurotransmission is required for ethanol-induced behavioral disinhibition.
Dopamine in behavioral disinhibition

- elevated DA
- blockade of DAT
- abnormal behavioral disinhibition
- enhanced extracellular DA levels
- abnormal behavioral disinhibition?

fumin mutant lacking DAT

- fumin transposon 100 insertion in dat: "truncated DAT -- non-functional"
  (Kume et al., J. Neuroscience 2005; Makos et al., Analytical Chemistry, 2009)
- fumin mutant’s locomotor behavior monitored by FlyTacker (a multiple object tracking system based on Kalman filter, collision handling)

Two facets of impulsivity

- impulse choice – measured by delay discounting task
- inhibitory failure – measured by go/no-go, stop signal reaction time, five-choice reaction time tasks

Alcohol Clin Exp Res. 2010; 34: 1306–1318

Presented at the 13th RCMI International Symposium on Health Disparities | December 9-13, 2012 | San Juan, Puerto Rico

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Go / No-Go test in human subjects

- test response inhibition
- measure trait impulsivity
- ADHD group – more errors

No-Go Test in Drosophila

- Test response inhibition, measure ~ trait impulsivity
- fumin mutant – loss of response inhibition ---- impulsivity

Characteristics of fumin’s impulsivity

- measure activity > 60 mm/sec per fly = hyperkinetic event
- Increase with more salient stimulus
Characteristics of fumin’s impulsivity

- Slightly less in females, but not significant
- Decrease with aging

fumin: impulsivity-modulating factors

- Fumin’s hyperkinetic activity: requires visual input
  - Under regular vs. infrared light

- Fumin’s hyperkinetic activity: sensitive to the presence of other flies
Neuromodulators
- dopamine

Behavior
- impulse control

Brain
- DA receptors (in particular neural sites)

Social stress
- Environment (positive/negative)

**Downstream effectors of DAT**

**Pharmacological blockade of DA receptors**

- D1 antagonist, but not D2 antagonist, blocks motor impulsivity in fumin mutants

**Graphs**

- Graphs showing the effect of D1 and D2 antagonists on motor impulsivity.
Genetic interaction of DAT & DA receptors

- dDA1 is a major receptor mediating motor impulsivity in *fumin* mutants.

Genetic interaction of DAT & DA receptors

- D2 may also be involved in motor impulsivity in *fumin* mutants.

Conclusions

- Flies lacking DAT (hyper dopamine tone)
  - Impaired response inhibition, representing motor impulsivity
  - Sensitive to visual input - not background, but salient stimuli - "social stress"
  - Require dDA1 (D1 dopamine receptor) for motor impulsivity
  - A sensible model for impulsivity associated with addiction as well as neurodevelopmental disorders such as ADHD, autism, schizophrenia, drug, etc.
  (gene x social x environmental factors)

Where? (neural substrates for cognitive vs. motor disinhibition)

How? (cellular factors and signaling pathways)
Can mapping brain circuits controlling eating behavior lead to treatments for obesity and diabetes?

Arshad M. Khan, Ellen M. Walker, Anais Martinez, Briana E. Pinales, Nicole Dominguez, Sarah D. Chenausky, Joshua Ortíz-Guzmán, Claire E. Wells and Teresia A. Carreon

UTEP Systems Neuroscience Laboratory
Department of Biological Sciences and Border Biomedical Research Center
University of Texas at El Paso

13th RCMI Biennial Meeting
San Juan, Puerto Rico
10-13 Dec 2012
Obesity and Diabetes are Epidemics with Health Disparities

- Mexican-American males, (12-19 yrs) had the highest prevalence of obesity (22.1%) compared to non-Hispanic white (17.3%) and black males (18.5%).
- Obesity in Mexican-American females increased from 9.2% to 19.9% from 1988-1994 to 2003-2006.
- 31.7% of all U.S. children (10-17 yrs) are overweight or obese; 40.9% of all Hispanic children in this age range are obese.

Data from the United States-Mexico Border Health Commission 2009 White Paper

- The new york times, today.
Metabolic complications can originate from brain circuit dysfunction

Hyperphagia, Rape, and Dementia Accompanying a Ventromedial Hypothalamic Neoplasia
Arndt Neumann—Vol 58, June 1969
Case History
• 20 year old Puerto Rican woman
• Bookkeeper by profession
• First admitted to New York Hospital in Nov. 1962
• Complained of suffering from polydipsia, polyuria, and bulimia for the past year
• No other behavioral deficits; no impaired memory; body x-rays normal; blood panel normal
• Suspected hypothalamic tumor

In July 1964, patient's behavior had changed markedly; she "became withdrawn but was given to frequent outbursts of unprovoked laughing, crying, and, at times, rage."

On 16 Oct 1964, underwent a second craniotomy and an inoperable tumor at the base of the brain was found

Although she was managed for metabolic disease, she died and a postmortem study was conducted.
Metabolic complications can originate from brain circuit dysfunction

Hyperphagia, Rages, and Dementia Accompanying a Ventrromedial Hypothalamic Neoplasm

Limitations of PET or fMRI

Research Goals of the UTEP System Neuroscience Laboratory (Established 2011)

1. Identify feeding & autonomic control circuits at high resolution
   • Glucose-sensing brain circuits in normal and diabetic animals
   • "Hunger circuits" contributing to overeating and obesity
   • Create high-resolution maps of brain regions activated by hypoglycemia

2. Identify pharmacologic targets for therapeutics
   • Pharmacologic targets for appetite and glycemic control
   • Pharmacologic targets for neural control of autonomic function
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   - Pharmacologic targets for appetite and glycemic control
   - Pharmacologic targets for neural control of autonomic function

3. Manipulate these circuits to feeding and glycemic control
   - Optogenetic tools to control behavior and autonomic function

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#### Optogenetic Control of Hypothalamic Neurons


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#### Research Highlight: Creating a High Resolution Atlas of the Mammalian Hypothalamus

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αMSH  
nNOS  
MCH  

calbindin  
MCH  
Hcrt/orexin  

Series 1: 0 μm  
Series 2: 20 μm  
Series 3: 40 μm  
Series 4: 60 μm  
Series 5: 80 μm  
Series 6: 100 μm
Conclusions

- Our initial goal is to map nerve cell populations involved in feeding and glycemic control.
- The long-term use of these maps will help us target brain cells for pharmacology and optogenetics.
- These maps will provide greater anatomical resolution of brain regions implicated clinically in patient fMRI and PET studies.
- Functional brain imaging data can be brought into anatomical register with our high resolution maps of the hypothalamus.

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**Additional Collaborators**
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- Manuel Miranda
- Csaba Fekete
- Luis de Leon
- Alan Watts
- Richard Thompson

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- Stanford University
- University of Southern California

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- NINDS – KO1
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Sex differences in cocaine-induced conditioned place preference

Stephanie K. Nygard
The CUNY Graduate Center
Hunter College, CUNY

Background

- Cocaine addiction causes millions of deaths every year
- Environment associated memories play a major role in cocaine addiction
- Males and females show sexually dimorphic responses to cocaine
Circuitry of Cocaine Addiction, Learning, and Memory

Aim: To investigate sex differences in signaling molecules extensively studied in the context of psychostimulant exposure after the acquisition of cocaine conditioned place preference

- Extracellular regulated kinase (ERK)
- cAMP response element binding protein (CREB)

Methods

Conditioned Place Preference (CPP)

Day 1 Day 2 Day 3 Day 4 Day 5 Day 6
Pre-test (drug-free) Saline Cocaine Saline Cocaine CPP Test (drug-free)
**CPP Behavior (drug-free)**

- CPP scores
- Locomotor Behavior

*Saline** □ **Cocaine

* *p < .05 difference between saline and cocaine of the same sex

* *p < .05 main effect sex

**Nucleus Accumbens**

- phosho-ERK
- phosho-CREB

Correlations between CPP score and protein levels

**Hippocampus**

- phosho-ERK
- phosho-CREB

Correlations between CPP score and protein levels

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Summary

General

- Cocaine induced CPP in males and females
- NAc and HIP pERK levels increased and were correlated to CPP scores
- NAc pCREB levels increased and were related to CPP scores

Summary

Sex-specific

- Cocaine females were more active during CPP test than males and saline controls
- HIP and PFC pERK levels were lower in saline females than saline males
- PFC pERK levels increased in cocaine females and were correlated to CPP scores
- PFC pCREB levels only increased in cocaine males
Conclusions

- The NAc was the only region that pERK and pCREB levels increased in males and females after cocaine CPP.
- The increased PfC and HIP pERK observed in cocaine females seem to derive from differences in basal levels.

Implications for health disparities and future directions

- Drug addiction research should include females.
  - Estrous cycle/hormone effects.
- Cocaine addiction treatment strategies should consider sex differences.

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Questions?

Thank you for participating!