Drug Addiction: From Mice to Men and Back

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Understanding behavior in health and disease
When things go wrong – genetics, environmental insults/stresses, injuries

- **Neurodegenerative diseases** - Alzheimer’s, Mild Cognitive Impairment, Parkinson’s Disease, Huntington’s Disease, ALS
- **Neuropsychiatric disorders** - depression, bipolar disorder, OCD, schizophrenia
- **Disorders of decision-making** - addiction, substance abuse, alcoholism, risky behaviors
- **Neurodevelopmental disabilities** - mental retardation, autism spectrum disorders, ADHD, seizure disorders
- **Brain injury and repair** - stroke, traumatic brain injury and spinal cord injury, neural prostheses - brain-machine interfaces
- **Immune, inflammatory, glial disorders** - multiple sclerosis, brain inflammation and infection, glioblastoma, chronic pain
Incidence and costs of Brain Disorders in the U.S. (2004-2005)

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Total # of cases (x millions)</th>
<th>annual cost (x billion $)</th>
</tr>
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<tbody>
<tr>
<td>Parkinson’s disease</td>
<td>1.0</td>
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<td>stroke</td>
<td>4.7</td>
<td>51</td>
</tr>
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<td>spinal cord injury</td>
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<td>100</td>
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<td>alcoholism</td>
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<td>50.0</td>
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The Dopaminergic System

Activation of the VTA results in the release of dopamine
Nicotine is addictive

Nicotine in tobacco
• Alters synaptic function
• Produces neural adaptations
• Changes behaviors
Binding of nicotine to nAChRs in the VTA results in the release of dopamine.
Addiction: the hijacking of the mesolimbic dopamine system

1. Nicotine enters the brain through the bloodstream
2. Ventral tegmental area
3. Neurons in the nucleus accumbens are stimulated by dopamine and send signals to the prefrontal cortex
4. The decision making center, the prefrontal cortex, is affected by information from the nucleus accumbens resulting in addictive behavior

α4β2 nicotinic receptors in the ventral tegmental area are stimulated causing the release of dopamine
Drug Dependence

1. Impaired control over use of the drug.
2. Strong desire (or ‘craving’) to take it.
3. Much time taken obtaining/using/recovering.
4. A high priority given to drug use relative to other activities.
5. Continued use of the drug despite the harm that it causes.
6. Development of tolerance to the effects of the drug.
7. Withdrawal symptoms on cessation of use.
## Nicotine-withdrawal Symptoms in Humans

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<th>Affective</th>
<th>Somatic (physical)</th>
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<td>Depressed mood</td>
<td>Bradycardia</td>
</tr>
<tr>
<td>Irritability</td>
<td>Insomnia</td>
</tr>
<tr>
<td>Anxiety</td>
<td>Gastrointestinal discomfort</td>
</tr>
<tr>
<td>Frustration</td>
<td>Increased appetite leading to weight gain</td>
</tr>
<tr>
<td>Difficulty concentrating</td>
<td>A general dysphoric state</td>
</tr>
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<td>Craving for tobacco</td>
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Tobacco and Nicotine Addiction
Nicotine Withdrawal Symptoms in Rodents

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<th>Non-somatic signs</th>
<th>Somatic Signs</th>
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<tbody>
<tr>
<td>Hyperalgesia</td>
<td>Head nodding</td>
</tr>
<tr>
<td>Anxiety-like responses</td>
<td>Wet dog shakes</td>
</tr>
<tr>
<td>Conditioned place aversion</td>
<td>Paw tremors</td>
</tr>
<tr>
<td>Elevated brain stimulation reward threshold</td>
<td>Excessive grooming</td>
</tr>
<tr>
<td></td>
<td>Excessive scratching</td>
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The Neuronal Nicotinic Receptor

11 genes encoding α & β nAChR subunits

ACh binding pocket

gate
The $\alpha_5$ and $\beta_4$ nAChR Subunits are Expressed in the MHb and IPN.

$\alpha_5$ In Situ hybridization

$\beta_4$ In Situ hybridization
α5 Subunit Necessary for Physical Manifestations of Nicotine Withdrawal

β4 Subunit Necessary for Physical Manifestations of Nicotine Withdrawal

β2 Subunit Not Necessary for Physical Signs of Nicotine Withdrawal

Microinjection of Mecamylamine Precipitates Withdrawal ONLY in MHB and IPN

Microinjection into Other Areas Does Not Elicit Withdrawal Behaviors

The MHb Comprises Cholinoceptive & Cholinergic Neurons

\[ \text{CHAT-ChR2} \]

\[ \text{vMHb} \]

\[ \text{125} \text{ I-Epibatidine} \]
Electrophysiological recordings in the MHB

- Coronal brain slices with MHB (250 µm)
- Whole-cell patch clamp electrophysiology
- Intrinsic Excitability
Nicotine Enhances Excitability of MHB Neurons

A) Nicotine Baseline
+50 pA
+40 pA
+30 pA
+20 pA
+10 pA

B) 2 s
LHb LHb
DG DG 3V
Nicotine Baseline

C) Initial 500 ms
Baseline
Nicotine

D) 2 s
Baseline
Nicotine

E) Initial 500 ms
Baseline
Nicotine

F) Mecamylamine
2 s
Baseline
Nicotine

G) Mecamylamine
Initial 500 ms
Baseline
Nicotine

Blocks nAChRs

Dao et al. (under revision)
Null Mutation of α5 nAChR Subunit Blocks Facilitation of Excitability by Nicotine

*Dao et al. (under revision)*
Neurokinin Receptor Antagonists Block Nicotine-induced Facilitation of Excitability

A) L-732,138

B) SB222200

C) L-732,138+SB222200

Dao et al. (under revision)
New Functional Pathway

α5- nAChRs

Substance P &/or NKB

IP$_3$

Gq

PLC

PKC

Raf

MEK

MAPK

[Ca$^{2+}$]$_{in}$

Smooth endoplasmic reticulum

Gene Regulation

ERK

Fos
Habenular microinjection of neurokinin receptor antagonists induces somatic signs of withdrawal in mice chronically treated with nicotine

Withdrawal Score

Vehicle | L-732,138 | SB222200 | L-732,138 +SB222200

Dang Dao & Erika Perez
Other Drugs?
α5- and β4-containing nAChRs Influence the Physical Manifestations of Morphine Withdrawal

![Graph showing physical manifestations of morphine withdrawal for Wild Type, α5 null, and β4 null mice.](image)

**p>0.01 Compared to Saline Control, #p>0.01 Compared to Wild type**

Erika Perez & Damaj lab
From Mice to Men.... & Women!
Single Nucleotide Polymorphism in the Human $\alpha_3 \alpha_5 \beta_4$ gene cluster
α5 Gene Variant Associated with Substance Abuse Alters Receptor Function

rs16969968 SNP "Mr. Big"

CHRNA5 3' 5'

D398N

Extracellular

Intracellular

(Bierut et al 2008)
The **CHRNA5/A3/B4 gene cluster** variability as an important determinant of **early alcohol and tobacco initiation** in young adults.


**Chromosome 15q25.1** genetic markers associated with level of response to alcohol in humans.
Geoff Jodym, Garry Brush, Margaret Robertson, Tom L. Smith, Jelger Kalmijn, Marc Schuckit, Raymond L. White.

**α-5/α-3 nicotinic receptor subunit** alleles increase risk for **heavy smoking**.

W Berrettini, X Yuan, F Tozzi, K Song, C Francks, H Chilcoat, D Waterworth, P Muglia, and V Mooser.


**Nicotinic Receptor Gene Variants Influence Susceptibility to Heavy Smoking**

Victoria L. Stevens, Laura J. Bierut, Jeffrey T. Talbot, Jen C. Wang, Juzhong Sun, Anthony L. Hinrichs, Michael J. Thun, Alison Goate, and Eugenia E. Calle.

Cancer Epidemiol Biomarkers Prev 2008;17(12). December 2008

**Association of a single nucleotide polymorphism in neuronal acetylcholine receptor subunit alpha 5 (CHRNA5) with smoking status and with ‘pleasurable buzz’ during early experimentation with smoking**

Back to Mice.......
Critical role of the α5 nAChR Subunit in Intravenous Nicotine Self Administration

Re-expression of α5 WT and α5 rs 16969968 in the ventral tegmental area

In collaboration with Fauvre & Maskos
Tobacco Smoking

• Causes 1/3 of cancer-related deaths in developed countries

• Global epidemic: 4-5 million annual deaths from smoking-related causes
  – Predictions: increase to 10 million annually by 2030

• Leading cause of preventable death worldwide

Vineis et al., 2004; Mathers et al., 2006; Giovino et al., 2012; Kabir et al., 2013
The *CHRNA5-A3* Region on Chromosome 15q24-25.1 Is a Risk Factor Both for Nicotine Dependence and for Lung Cancer

Margaret R. Spitz, Christopher I. Amos, Qiong Dong, Jie Lin, Xifong Wu

*Brief Communication | JNCI*  
*Vol. 100, Issue 21 | November 5, 2008*
Conclusions part 1

The MHb is a fundamental structure for the expression of the physical signs of nicotine and ethanol withdrawal.

\( \alpha_5^* \) and \( \beta_4^* \) nAChRs are necessary for the manifestations of the physical signs of abstinence from nicotine and, in part, from morphine.
Conclusions part 2

The MHb acts as a gatekeeper in the control of drug consumption, especially of doses that are normally aversive.

The contribution of α5 and β4 subunits is critical for this function.
Conclusions part 3

Mouse models and human genetics can work hand in hand to address the molecular mechanisms of disease.
Acknowledgements

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• Yanfen Teng, Ph.D.

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• Kazu Oka’s Lab

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