Forgiving the Sins of the Father: Epigenetic Inheritance of a Cocaine Resistance Phenotype

Chris Pierce
Center for Neurobiology and Behavior
Perelman School of Medicine
University of Pennsylvania
Currently over 2 million regular cocaine users in the United States
Currently over 2 million regular cocaine users in the United States

- It is estimated that there are approximately 250,000 cocaine addicts in Chile.
- If these estimates are correct, the percentage of regular cocaine users in Chile is double that of the U.S., which is likely due to increased availability.
- Rates of cocaine addiction are strikingly high in northern Chile (upwards of 20% in some regions).
Animal model of cocaine addiction
Self-Administration
Epigenetics
Epigenetics

Inherited changes in phenotype caused by mechanisms other than changes in the associated DNA sequence
Nearly two meters of DNA are packaged into the nucleus.
Nearly two meters of DNA are packaged into the nucleus.

The diameter of a neuron’s nucleus is around 1-2 microns.
How does DNA fit into the nucleus?
How does DNA fit into the nucleus?

- DNA is VERY thin (width of around 2 nanometers).
How does DNA fit into the nucleus?

- DNA is VERY thin (width of around 2 nanometers).
- DNA is tightly wrapped around proteins called histones.
DNA and histones together are known as a **nucleosome**.
The nucleosome

- 146 base pairs of DNA are wrapped almost twice around an octamer of histones.
- There are five histones: H₁, H₂A, H₂B, H₃, H₄.
Histone, nucleosome, chromatin

- **Histone**: Proteins found in the nucleus that form the “spool” that DNA wraps around.
- **Nucleosome**: A single section of DNA wrapped around a collection of eight histones.
- **Chromatin**: All DNA and histones in the nucleus.
Chromatin can be so tightly packed that transcription cannot take place

- **Heterochromatin**: Closed chromatin – transcription silenced.
- **Euchromatin**: Open chromatin – ready for transcription.
Modifications of histone tails regulate chromatin structure (heterochromatin/euchromatin)
For example, histone methylation typically results in the heterochromatin form, which suppresses gene transcription.

During development some genes are permanently silenced via methylation (e.g. liver-specific genes in cells destined to become neurons).
Histone N-terminal tail modifications

- Methylation
- Acetylation
- Phosphorylation
- Ubiquitination
- Sumoylation
- Ribosylation
Histone N-terminal tail modifications determine transcription factor binding

- Methylation (silence – heterochromatin)
- Acetylation (activate - euchromatin)
Chromatin remodeling and disease

- **Genetic diseases**
  - Rhett syndrome (autism-like)
    - Mutation in X-linked MECP2 (methyl CpG binding protein 2) gene altering chromatin *methylation*
  - Rubinstein-Taybi syndrome (learning difficulties)
    - Mutation of CREB binding protein gene altering chromatin *acetylation*

- **Environmental diseases**
  - Cancer
    - e.g. DNA methylation causing loss of expression of tumor suppressor genes
  - Addiction?
Cocaine-induced chromatin remodeling increases BDNF transcription in the rat mPFC

Sadri-Vakili, Kumaresan, Schmidt, Famous, Chawla, Vassoler, Overland, Xia, Bass, Terwilliger, Pierce & Cha

14 Days Cocaine Self-Administration (or Yoked Saline) 7 Days Forced Abstinence
Cocaine self-administration increases BDNF protein in the mPFC

14 Days Cocaine Self-Administration (or Yoked Saline)  7 Days Forced Abstinence

BDNF (%GAPDH)

saline  cocaine

+3.20

*
BDNF=brain-derived neurotrophic factor

- Several studies have shown that BDNF plays an important role in cocaine-induced behavioral and neuronal plasticity.
- Cocaine-induced changes in BDNF protein likely result from changes in BDNF transcription and associated epigenetic alterations.
BDNF=brain-derived neurotrophic factor

- The rodent BDNF gene has 8 promoters driving the transcription of 8 exons, which are spliced to a common 3’ coding exon.
- These promoters are differentially regulated.
- All exons code the same BDNF protein product.

- **Promoter:** A region of DNA that facilitates the transcription of a particular gene.
- **Exon:** A sequence of DNA that codes information for protein synthesis that is transcribed to mRNA.
Cocaine self-administration increases BDNF mRNA in the mPFC

14 Days Cocaine Self-Administration (or Yoked Saline)  7 Days Forced Abstinence

BDNF Transcript (ng)

- Saline
- Cocaine

* Indicates significant difference
Gene expression requires two steps

- Chromatin remodeling, which requires the modification of histone proteins
  - Association of chromatin elements with promoters can be assessed with Chromatin ImmunoPrecipitation (ChIP)
Cocaine self-administration increased AcH3 association with BDNF exon IV in the mPFC.
Gene expression requires two steps

- Chromatin remodeling, which requires the modification of histone proteins
- Sequence-specific transcription factor binding to promoter regions
BDNF transcription factors include:

- CREB, MeCP2
- CaRF, USF, Sp1, C/EBP and AP-1
Cocaine self-administration increased pCREB association with BDNF exon IV in mPFC
Cocaine self-administration decreased MeCP2 association with BDNF exon IV in mPFC
Molecular mechanisms underlying cocaine-induced increases in mPFC BDNF expression

- Increased association of AcH3 with BDNF exon IV
- Increased association of pCREB with BDNF exon IV
- Decreased association of MeCP with BDNF exon IV
Do decreases in mPFC BDNF expression influence cocaine self-administration?

- Microinjection of BDNF shRNA into mPFC
- shRNA targeted exon IX
- Collaborators: Caroline Bass & Ernie Terwilliger, Children’s Boston

<table>
<thead>
<tr>
<th>Time</th>
<th>AAV-GFP</th>
<th>AAV-GFP + BDNF shRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days FR1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days PR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Lever Pressing for Cocaine
BDNF shRNA produced an approximately 60% decrease in protein expression
Decreasing BDNF expression in the mPFC enhanced the reinforcing efficacy of cocaine
These results indicate that cocaine-induced increases in mPFC BDNF transcription represent a compensatory adaptation that reduces cocaine reinforcement.
Epigenetics

Inherited changes in phenotype caused by mechanisms other than changes in the associated DNA sequence
Epigenetics

Inherited changes in phenotype caused by mechanisms other than changes in the associated DNA sequence
Epigenetic Inheritance of a Cocaine Resistance Phenotype

60 Days of Cocaine Self-Administration or Yoked Saline

Breed with Cocaine Naïve Females

Examine Acquisition of Cocaine Self-Administration in Progeny
F0 Generation

F1 Cocaine Sired

F1 Saline Sired
Normal acquisition of cocaine self-administration in the female progeny of cocaine exposed fathers.
Delayed acquisition of cocaine self-administration in the male progeny of cocaine exposed fathers.
Delayed acquisition of cocaine self-administration in the male progeny of cocaine exposed fathers. Why?
Delayed acquisition of cocaine self-administration in the male progeny of cocaine exposed fathers. Why? Are they stupid?
Delayed acquisition of cocaine self-administration in the male progeny of cocaine exposed fathers. *Why? Are they stupid?*

SalSired and CocSired rats acquire food self-administration at similar rates, indicating no operant learning deficits.
Delayed acquisition of cocaine self-administration in the male progeny of cocaine exposed fathers. **Why?**
Delayed acquisition of cocaine self-administration in the male progeny of cocaine exposed fathers. Why? Is cocaine less reinforcing?
Reinforcing efficacy of cocaine reduced only in male cocaine sired rats. The male offspring of cocaine exposed sires rats will not work as hard for cocaine.

<table>
<thead>
<tr>
<th>Cocaine (mg/kg)</th>
<th>Breakpoint (Lever Presses/Session)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>SalSired</td>
</tr>
<tr>
<td>1.0</td>
<td>CocSired</td>
</tr>
</tbody>
</table>

![Graph of Breakpoint (Lever Presses/Session) vs. Cocaine (mg/kg)](image)

*Denotes significant difference compared to SalSired group.
Delayed acquisition of cocaine self-administration in the male progeny of cocaine exposed fathers. Why? BDNF plasticity?
Delayed acquisition of cocaine self-administration in the male progeny of cocaine exposed fathers. Why? BDNF plasticity?

Cocaine self-administration and BDNF

- Cocaine self-administration increases BDNF mRNA and protein in the medial prefrontal cortex.
- This appears to be a compensatory mechanism that decreases the reinforcing efficacy of cocaine.
Delayed acquisition of cocaine self-administration in the male progeny of cocaine exposed fathers. **Why?**
BDNF plasticity?
Delayed acquisition of cocaine self-administration in the male progeny of cocaine exposed fathers. Why? BDNF plasticity?

Male CocSired rats have increased mRNA and protein in the mPFC, which may decrease cocaine reinforcement (i.e. CocSired rats may like cocaine less).
What is the mechanism of cocaine-associated information transmission from father to son?
What is the mechanism of cocaine-associated information transmission from father to son?

Increased acetylation of BDNF promoters in sperm of cocaine exposed sires. These data indicate that cocaine can reprogram the sperm epigenome.
Male progeny of cocaine exposed fathers

- Delayed acquisition of cocaine self-administration.
- Operant learning intact.
- Increased BDNF protein and mRNA in mPFC, which may reduce cocaine reinforcement.

- Increased acetylation of BDNF promoters in sperm of cocaine exposed sires, which indicates that cocaine can reprogram the sperm epigenome.
Acknowledgements

- Lisa Briand
- Thomas Hopkins
- Blake Kimmey
- Pavel Ortinski
- Rachel Schassburger
- Heath Schmidt
- Ghazaleh Sadri-Vakili
- Fair Vassoler
- Samantha White
- Jenn Xue