Heritable Alterations in the Epigenome and BDNF Expression in Response to Self-administration or *in utero* Exposure to Cocaine in Rodents

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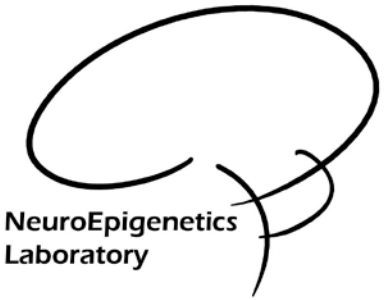
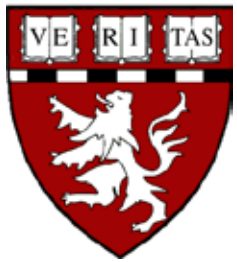




Introduction

- Effects of cocaine exposure are not limited to the individuals exposed to cocaine.
- Transmitted to offspring even though these subsequent generations were ***never*** exposed to cocaine.
- ***Significant*** public health and public policy ramifications.
- Population at risk from cocaine's effects may be much ***larger*** than currently recognized.

What we know:

- ✓ Genetic factors contribute significantly to the risk of cocaine abuse in humans.
- ✓ Animal models of addiction demonstrate that drugs cause epigenetic alterations in gene expression that can influence brain development and/or behavior.
- ✓ Trans-generational epigenetic alterations in gene expression influence behavior and are inherited across multiple generations *(exposure to chemicals, diet, stress)*
- ✓ Epigenetic alterations persist beyond the F1 generation: epigenetic modifications are incorporated into the germline.

Hypothesis:

Cocaine-induced alterations in the brain are heritable across multiple generations.

- ✓ Characterize the ***behavioral***, ***cellular***, and ***molecular*** events that may underlie the transmitted phenotype across multiple generations associated with exposure to cocaine in rodent models.

Collaborators

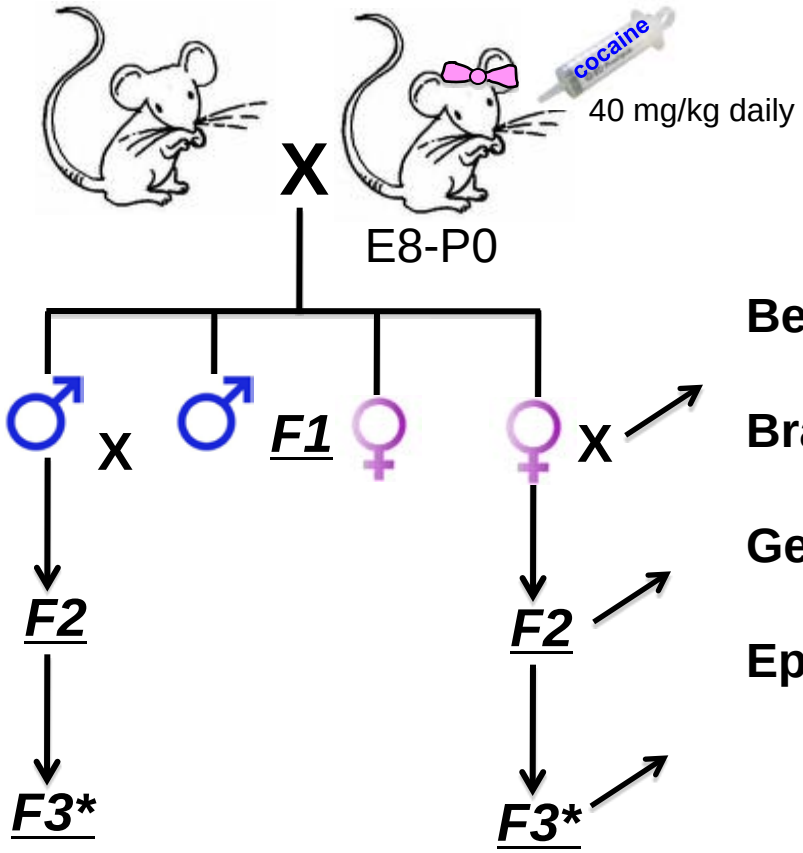
“Team Maternal”

+ Jinmin Zhu

“Team Paternal”

Transgenerational inheritance: Two experimental models

♀ Maternal transmission
in utero exposure



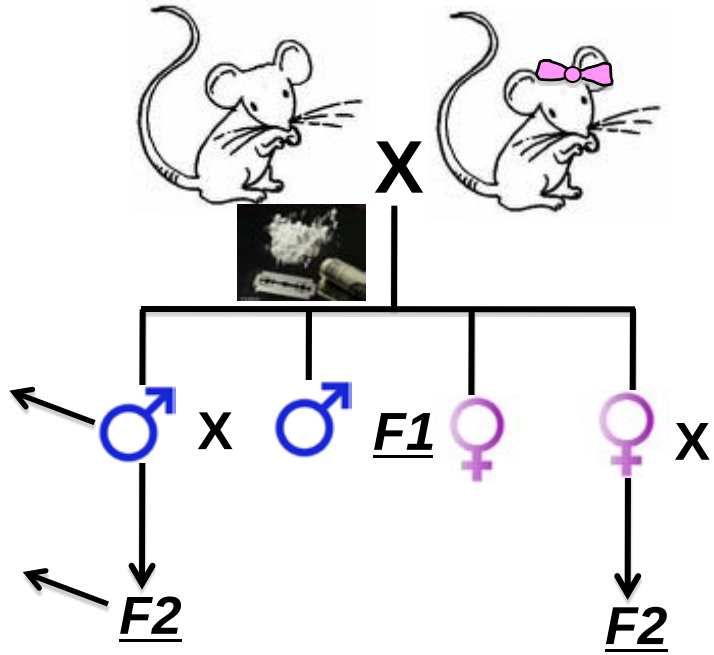
Behavioral Tests

Brain Analysis

Gene Expression

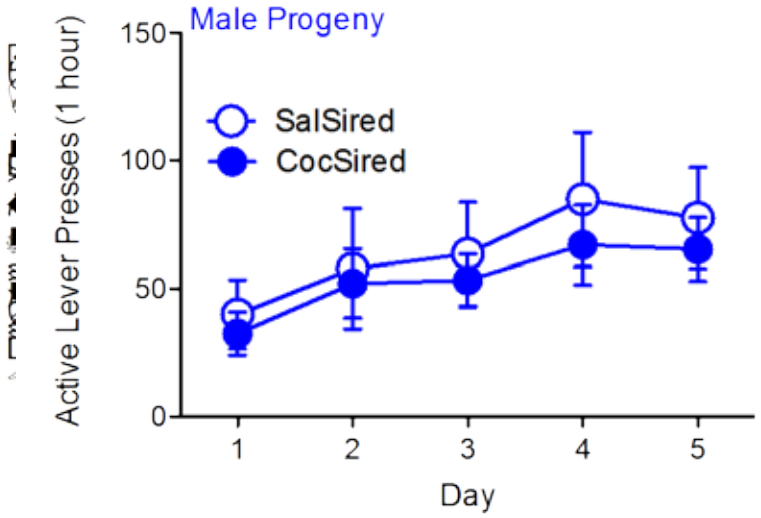
Epigenetic Marks

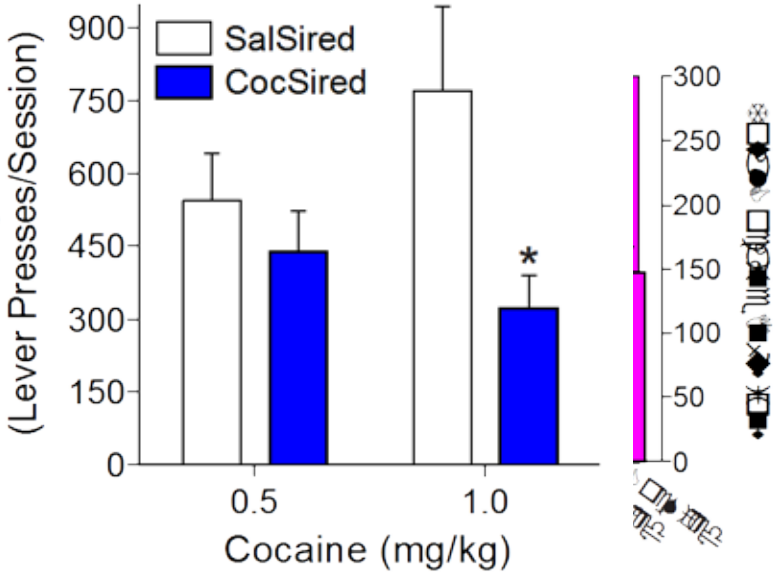
♂ Paternal transmission
Cocaine self-administration

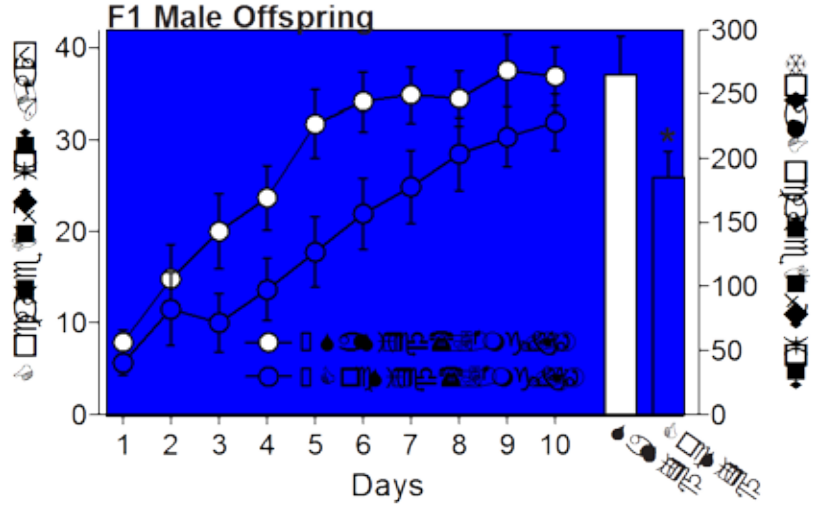


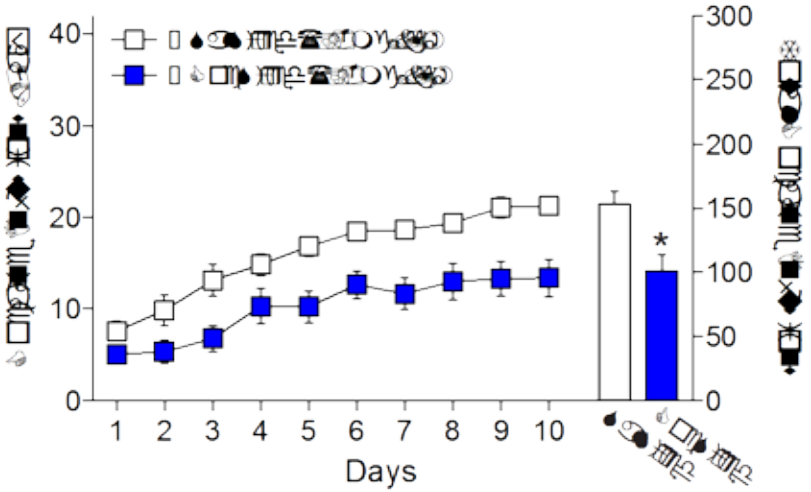
* The 3rd generation most important to demonstrate that the phenotype is transgenerational

Cocaine is less reinforcing in male offspring of CocSired rats









Why is cocaine less reinforcing in the male progeny?

1. Cocaine self-administration increases BDNF mRNA (exon IV-containing transcript) and protein in the medial prefrontal cortex (*Sadri-Vakili et al., 2010).*

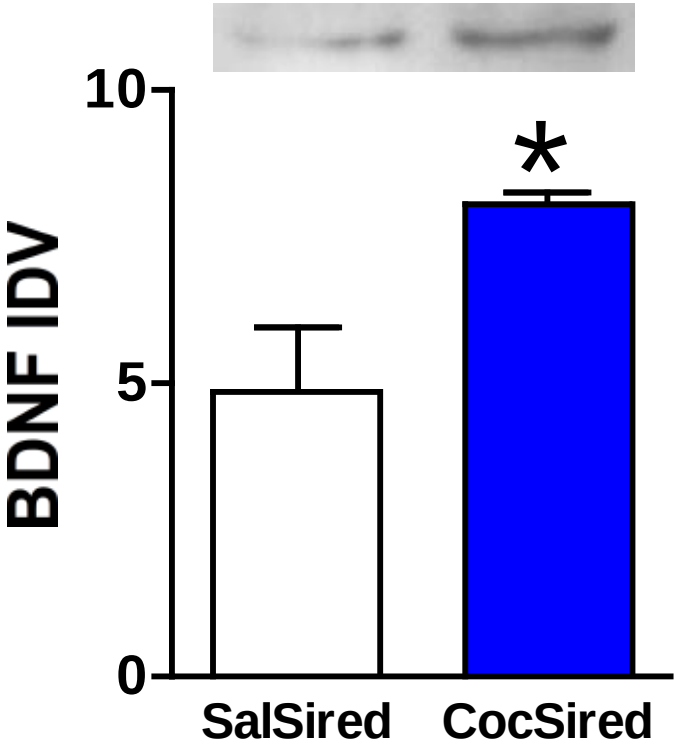
2. This appears to be a compensatory mechanism that decreases the reinforcing efficacy of cocaine (*Sadri-Vakili et al., 2010).*

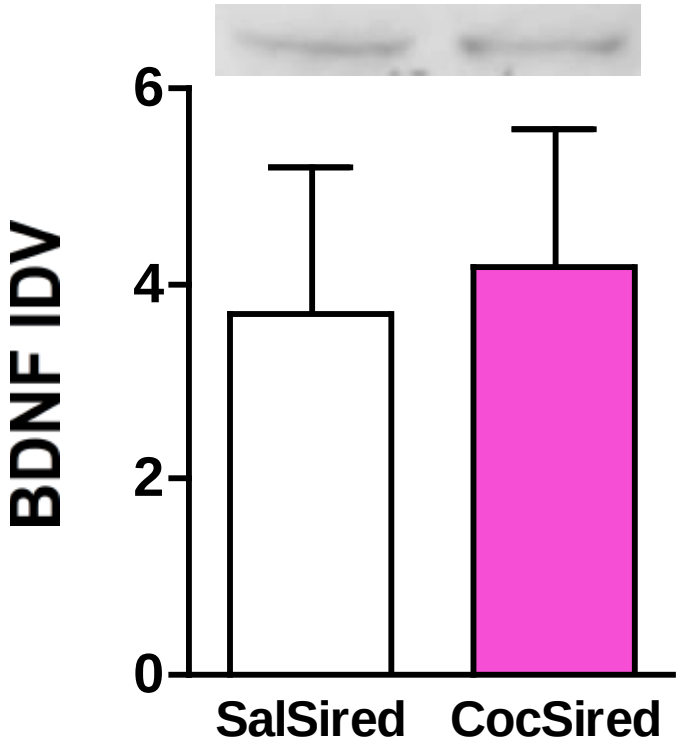
3. Infusions of BDNF into PFC suppresses cocaine seeking *(Berglind et al., 2007, 2009, 2011)*.

Hypothesis:

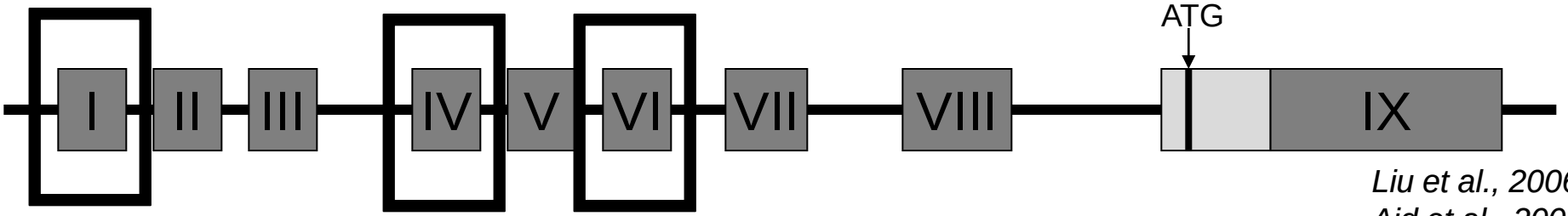
Decreases in reinforcing effectiveness of cocaine among male progeny of CocSired rats may be due to increased BDNF in the mPFC.

Increase BDNF protein levels in PFC of CocSired males

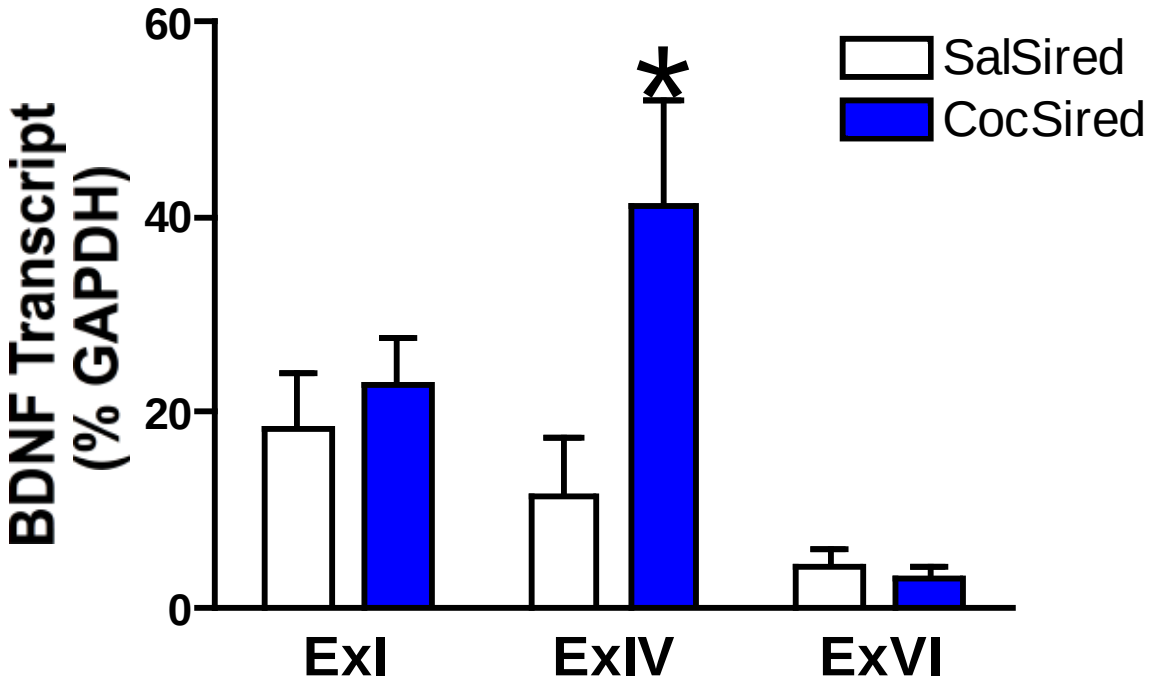




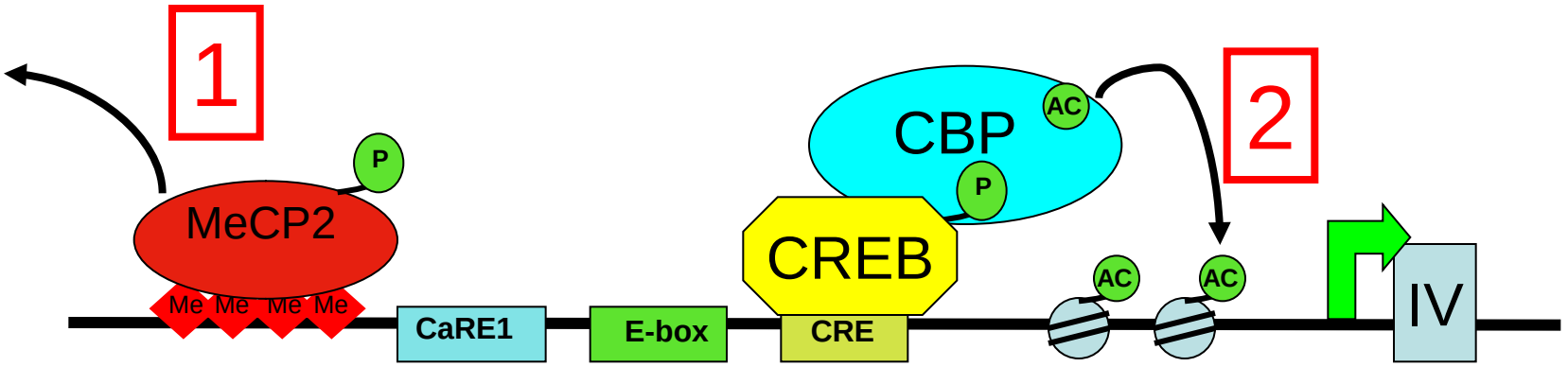
Increased BDNF exon IV-containing transcript in PFC of CocSired male rats



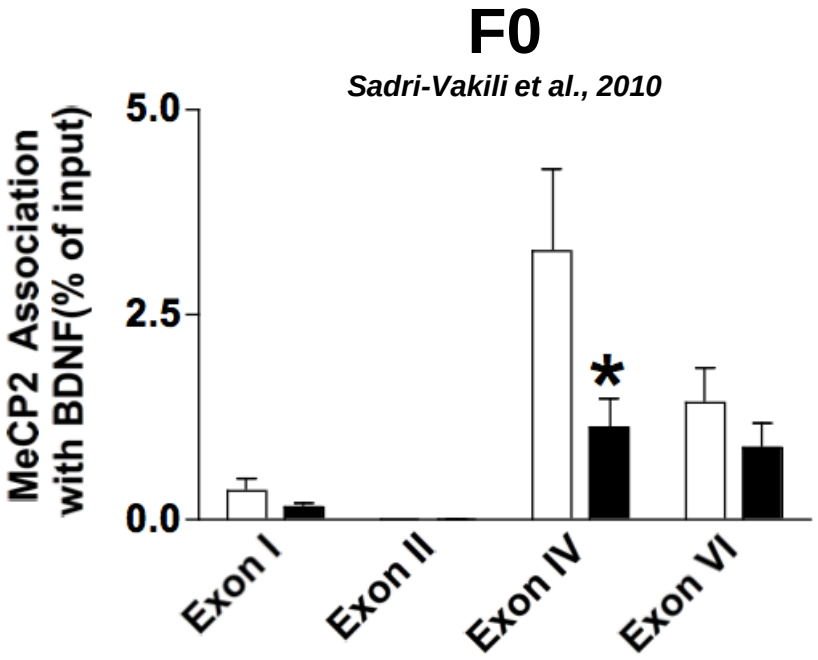
Liu et al., 2006
Aid et al., 2007

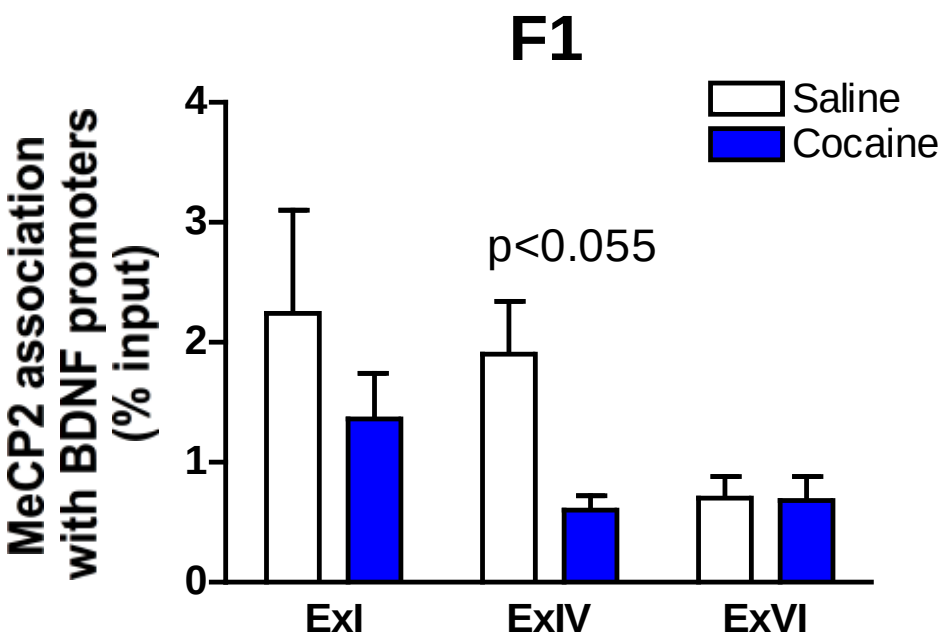


BDNF gene expression regulation in the PFC



Tao et al., 1998; West et al., 2001; Chen et al., 2003

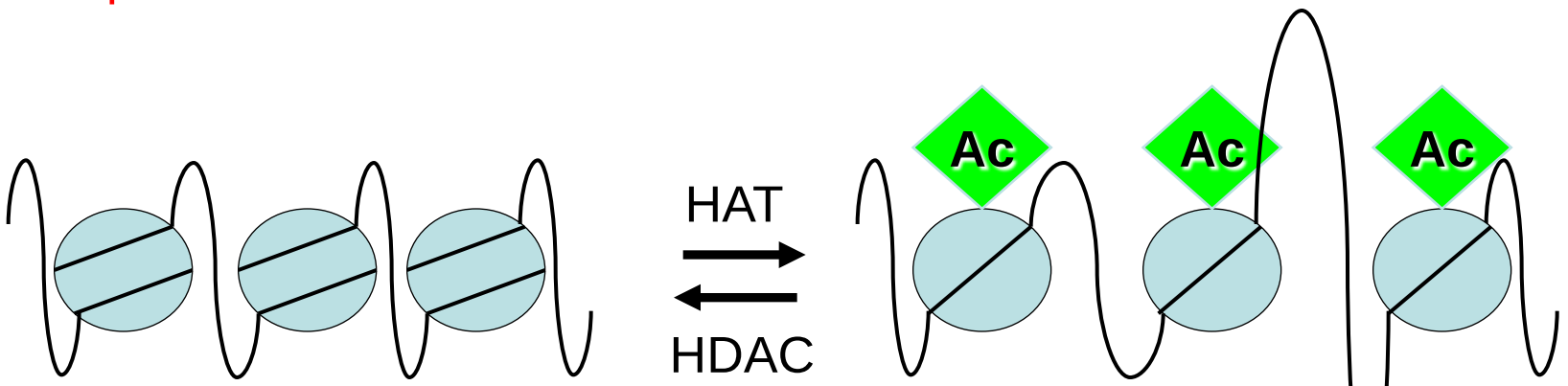




♂ BDNF gene expression regulation in the PFC

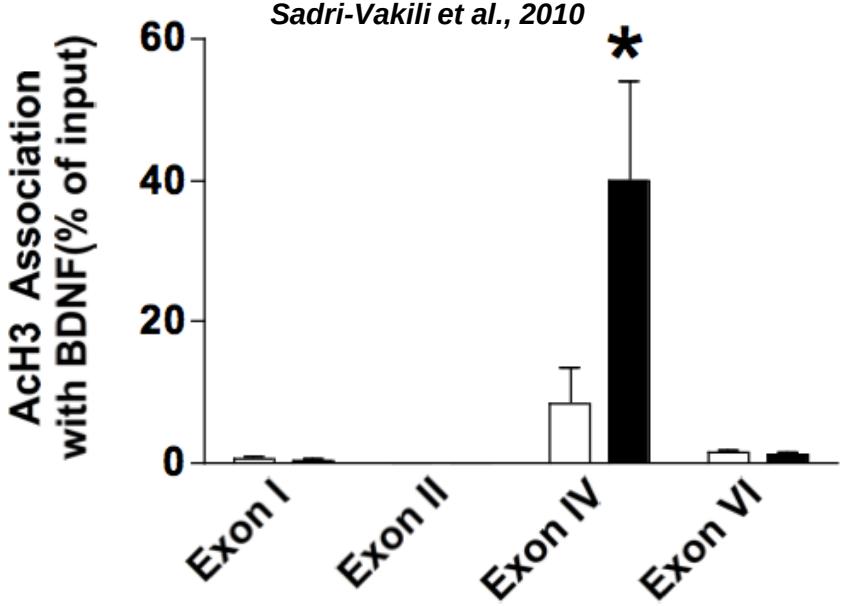
repressed chromatin

active chromatin

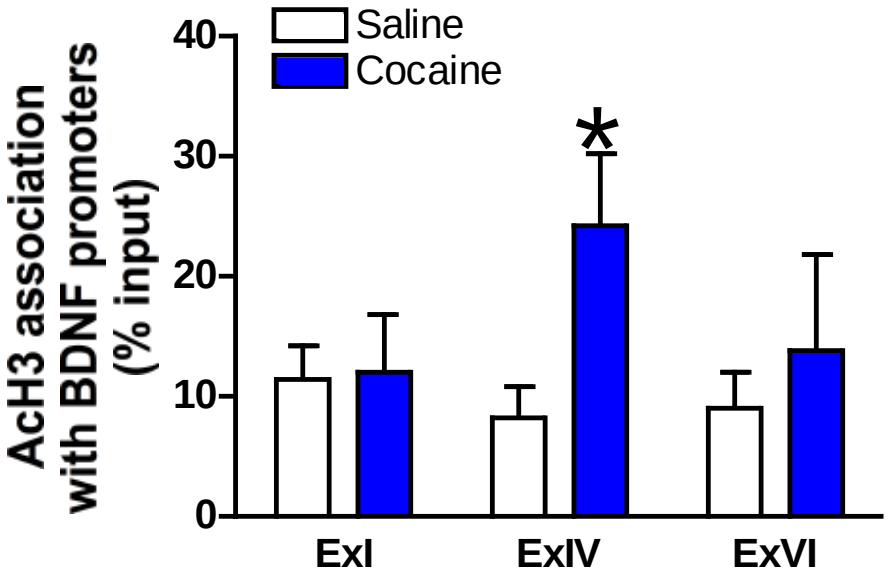


F0

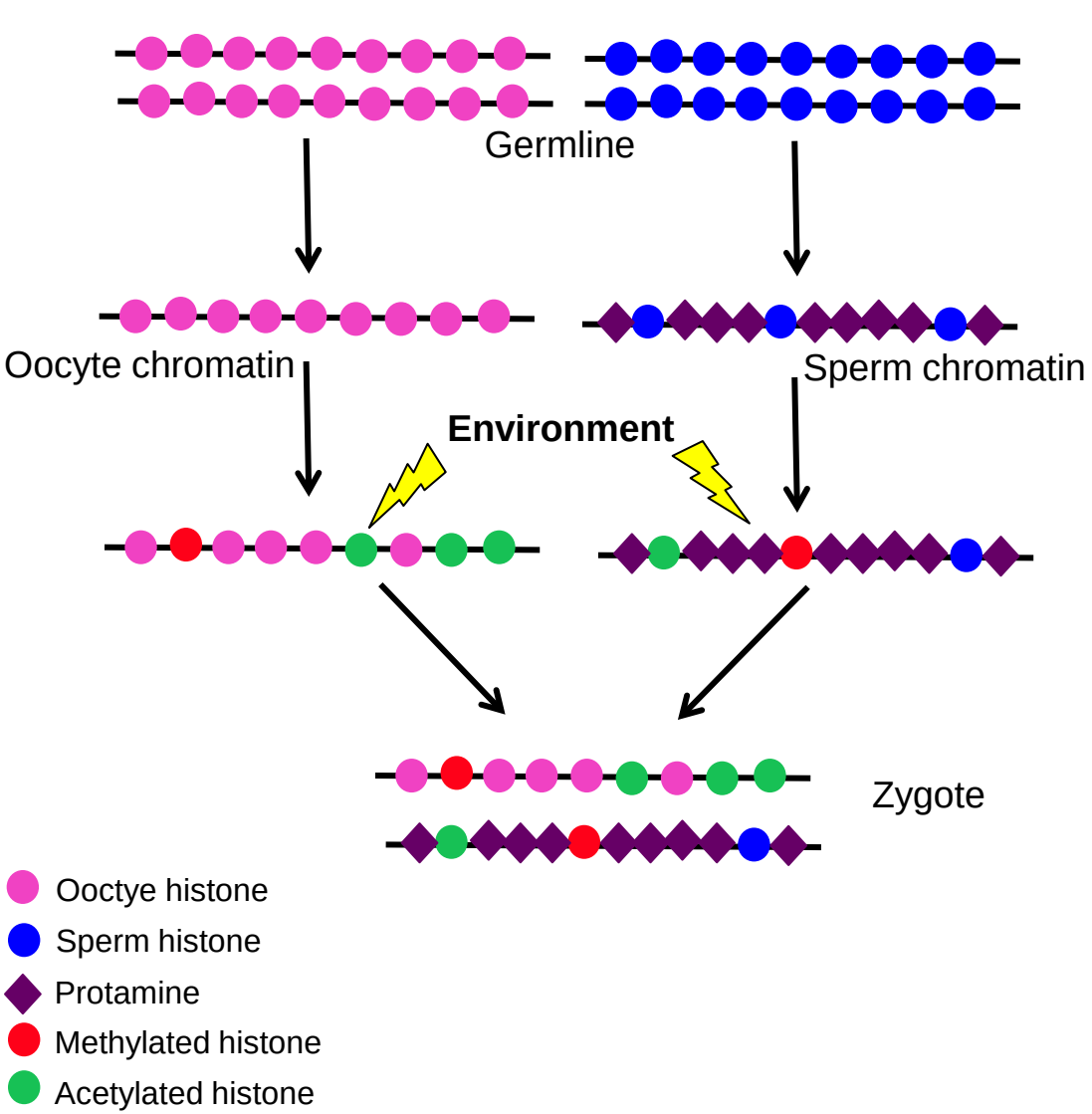
Sadri-Vakili et al., 2010



F1



What are the underlying mechanisms that transmit information from father to son?



Packaging of DNA in sperm:

✓Protamines

✓Histones

Epigenetic modifications in sperm:

✓DNA methylation

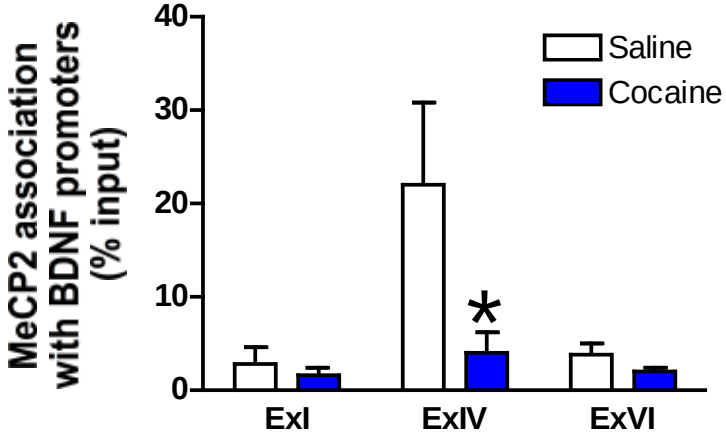
✓Histone methylation

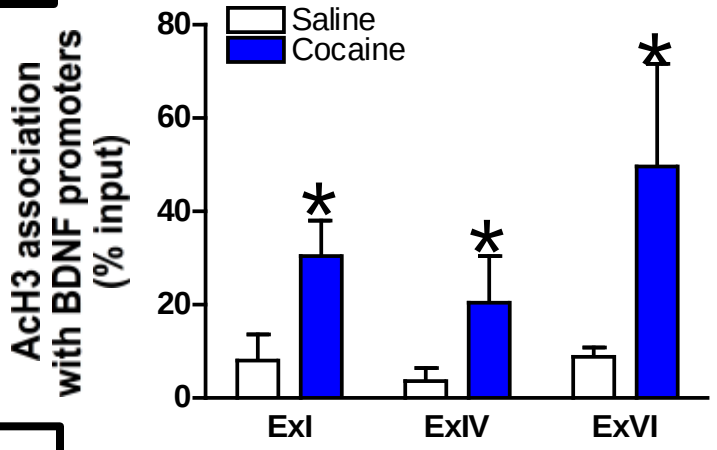
✓Histone acetylation

Hammoud et al., 2009; Steger et al., 2010; Puri et al., 2010

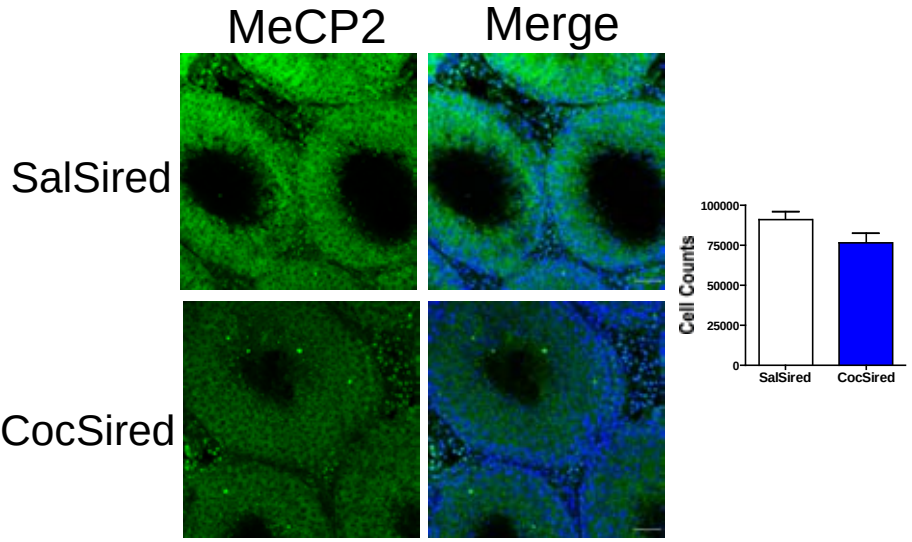
What are the underlying mechanisms that transmit information from father to son?

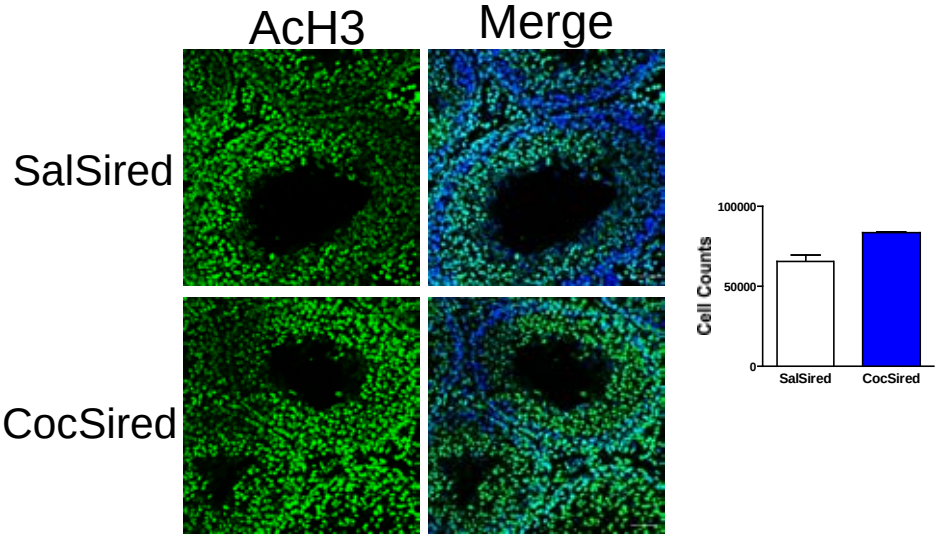
F0 Sperm





F1 Testes





Summary of Paternal Studies

- Delayed acquisition and reduced maintenance of cocaine self-administration in CocSired male rats

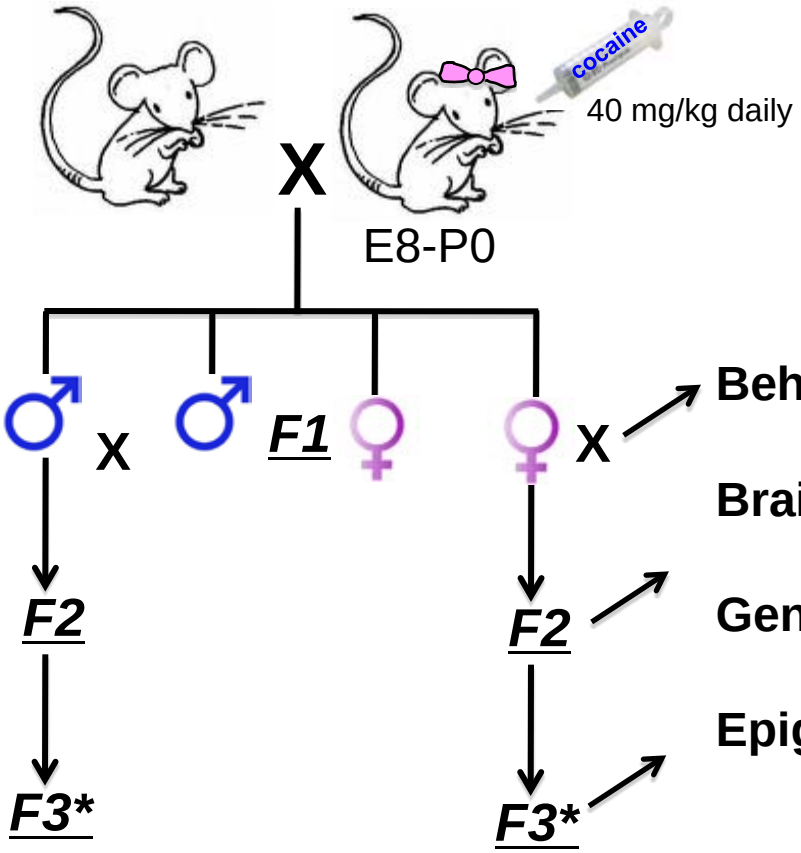
- Increased BDNF protein and mRNA in mPFC, which may reduce cocaine reinforcement.

- Epigenetic mechanisms:
  - Increased AcH3 at BDNF promoters in PFC and sperm
  - Decreased MeCP2 binding at BDNF promoters in PFC and sperm

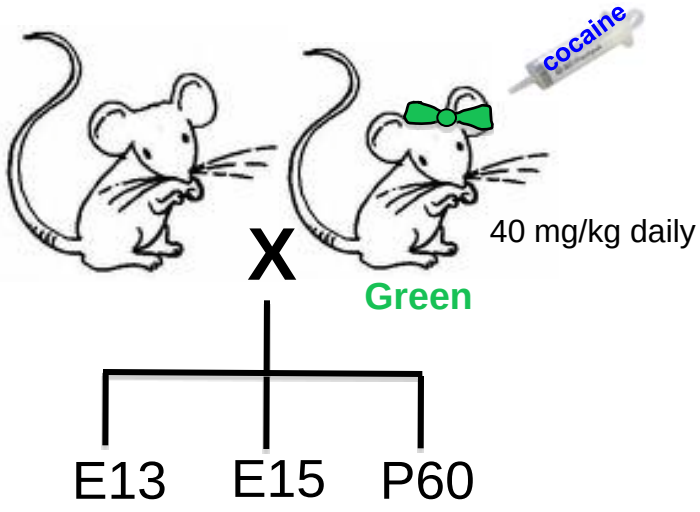
- Alterations in BDNF levels and epigenetic marks are heritable.

Maternal model

Maternal transmission
***in utero* exposure**



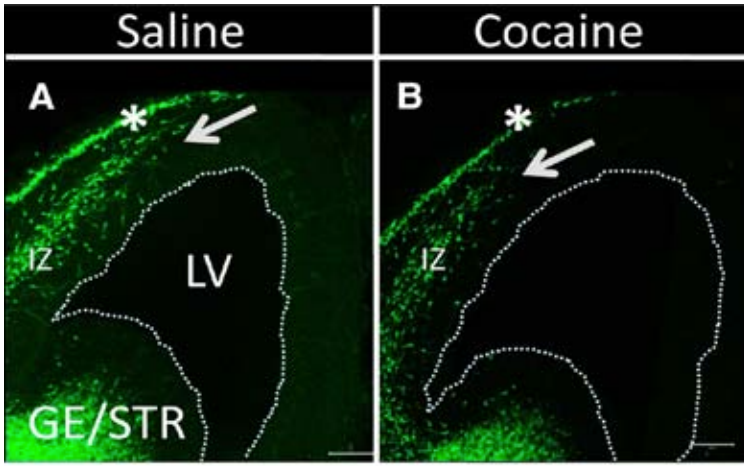
Heterozygote Swiss Webster
GAD67-GFP knock-in mice
(Tamamaki et al.,2003)

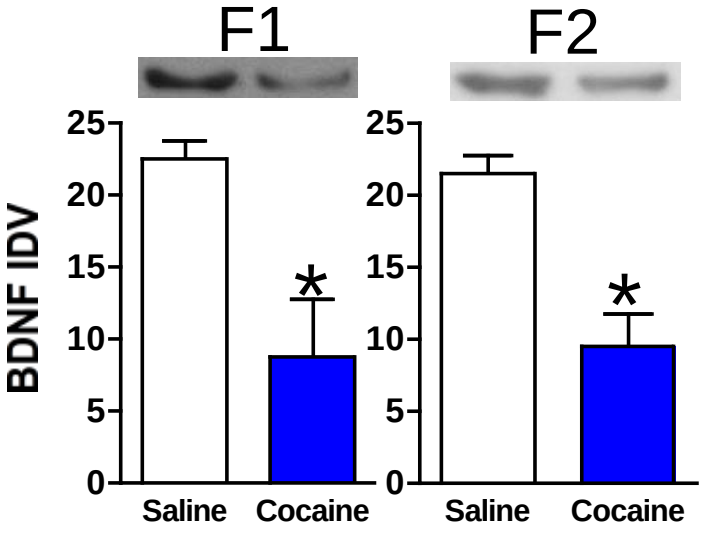


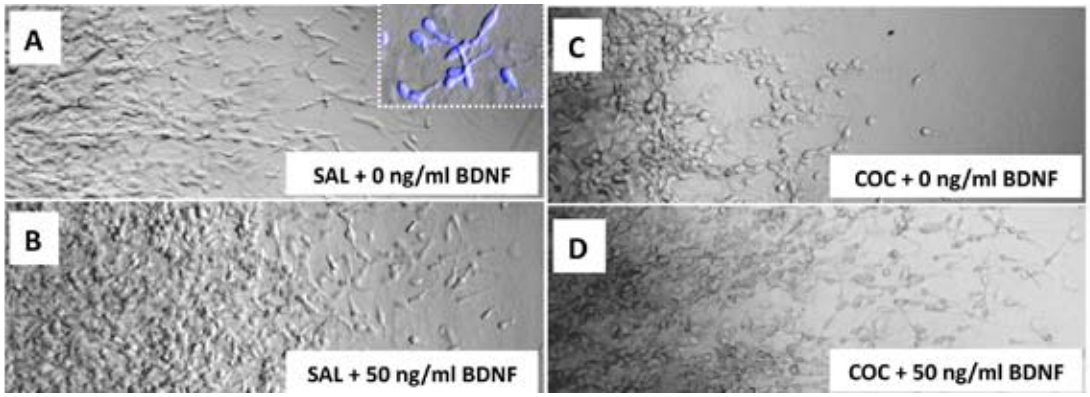
* The 3rd generation most important to demonstrate that the phenotype is transgenerational

BDNF-dependent decrease in GABA neuron migration in response to *in utero* cocaine

E13

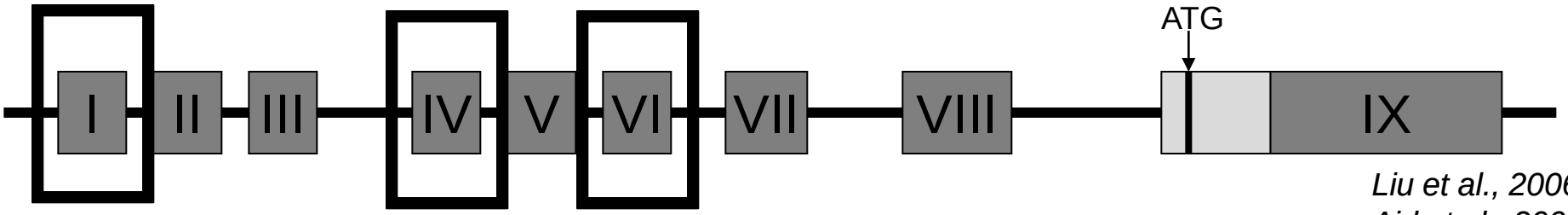




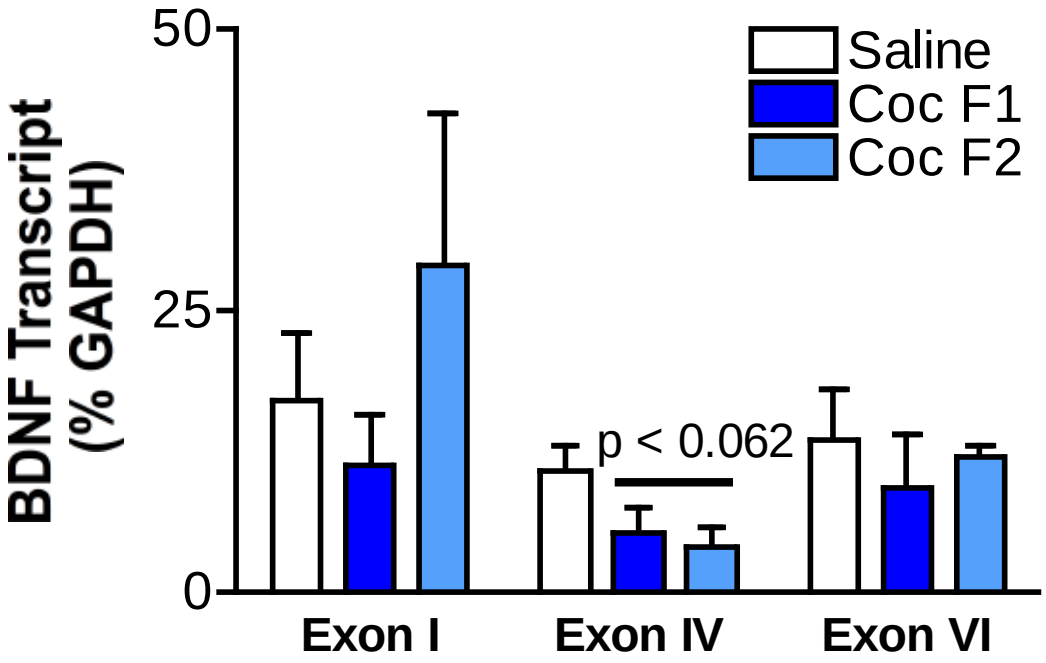


McCarthy et al., 2011, J. Neuroscience

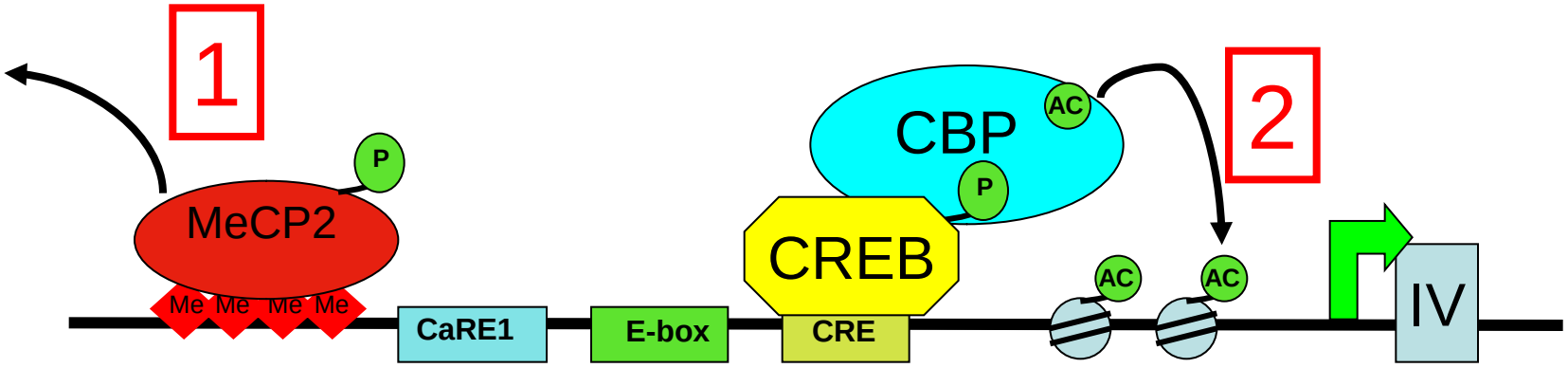
Cocaine-induced alterations in BDNF transcript levels are heritable



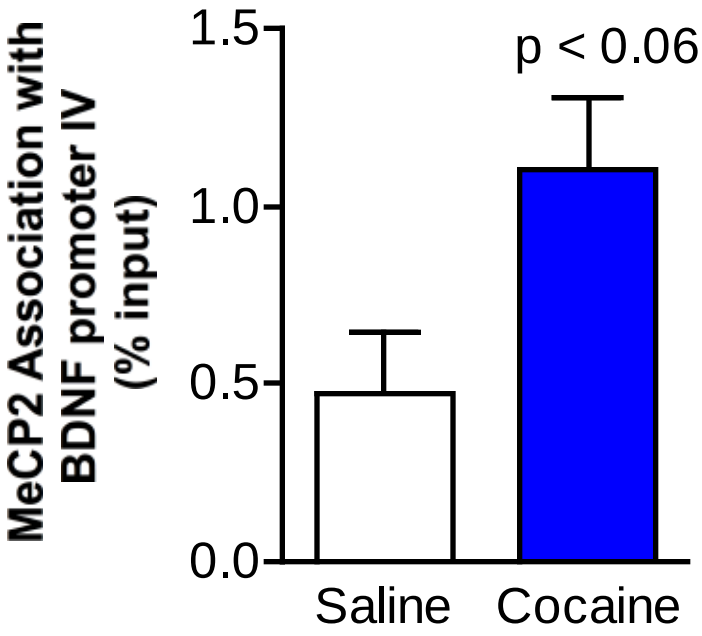
Liu et al., 2006
Aid et al., 2006

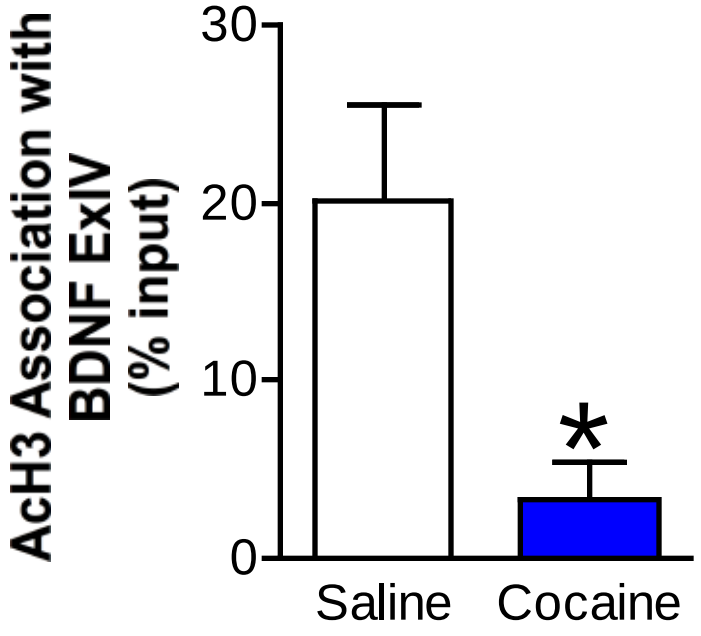


BDNF gene expression at E15 is regulated by:



Tao et al., 1998; West et al., 2001; Chen et al., 2003



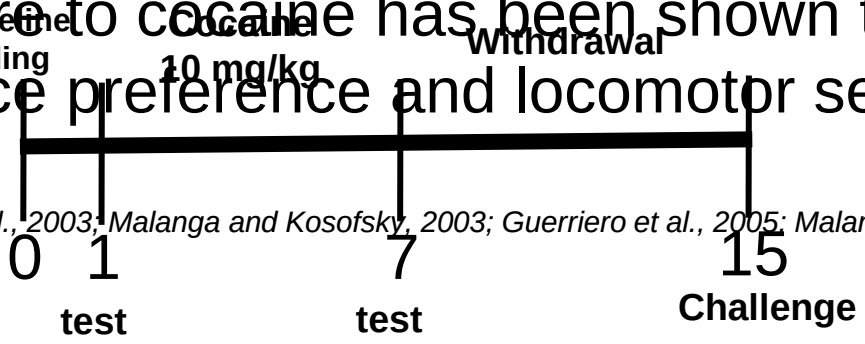


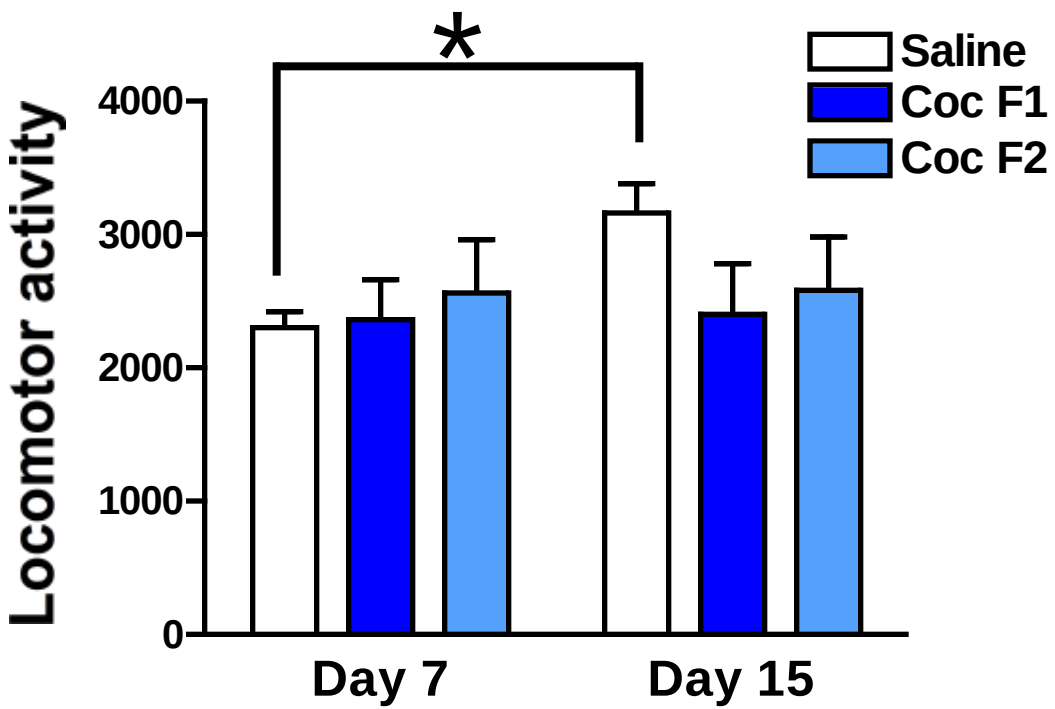
- What are the effects in the adult brain?
- Are there any behavioral consequences?

Prenatal cocaine exposure alters cocaine-induced locomotor sensitization in F1 and F2 generations

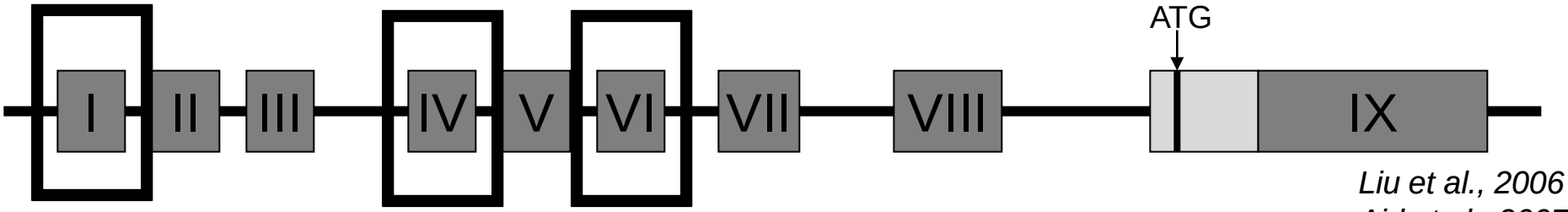
In utero exposure to cocaine has been shown to alter both conditioned place preference and locomotor sensitization to cocaine

(Rocha et al., 2002; Crozatier et al., 2003; Malanga and Kosofsky, 2003; Guerriero et al., 2005; Malanga et al., 2007; Malanga et al., 2008)





Heritable alterations in BDNF levels in the PFC at P60 in response to *in utero* exposure to cocaine



Liu et al., 2006
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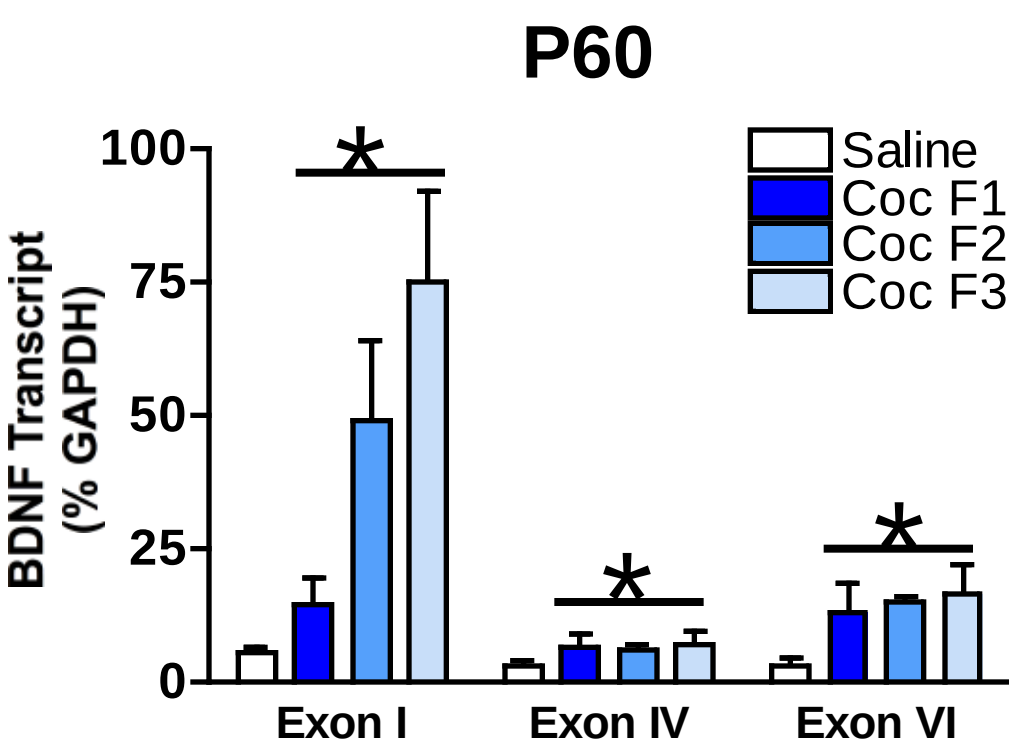

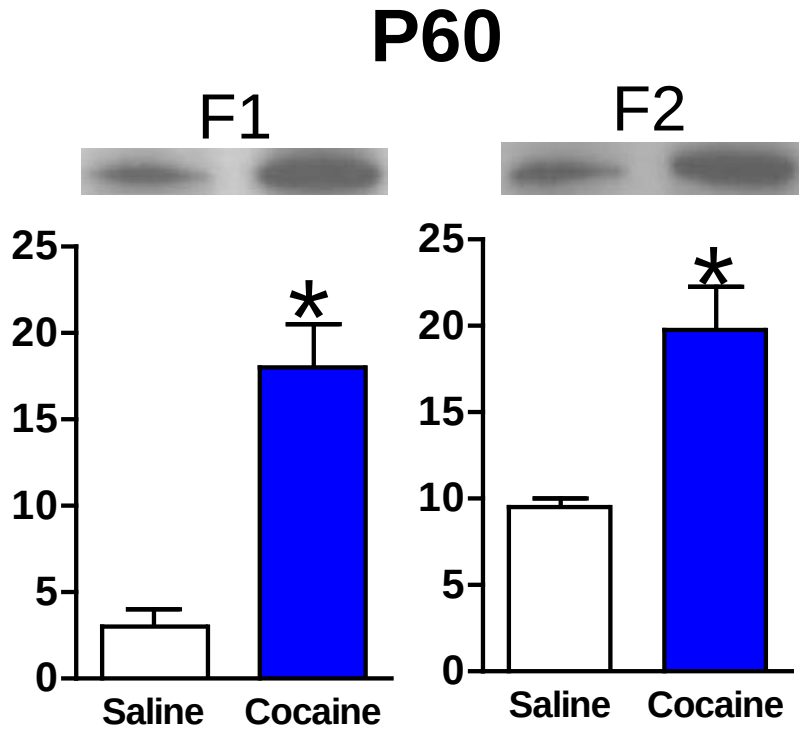

Summary of Maternal Studies

- Decreased GABA neuron migration during development in F1 and F2

- Heritable alterations in PFC BDNF protein and mRNA in embryos and adults
  - Decreased BDNF at E15
  - Increased BDNF at P60

- Molecular/epigenetic mechanisms that regulate BDNF:
  - Decreased AcH3 and increased MeCP2 at E15
  - Increased AcH3 at P60

- Cocaine exposure inhibits locomotor sensitization to cocaine in both F1 and F2 generations.

Comparison between "Paternal" and "Maternal" transgenerational studies (adults only)

Paternal

- ✓ Increased BDNF protein and mRNA in PFC
- ✓ Increased histone H3 acetylation
- ✓ Decreased MeCP2
- ✓ Decreased cocaine self-administration

Maternal

- ✓ Increased BDNF protein and mRNA in PFC
- ✓ Increased histone H3 acetylation
- ✓ Decreased MeCP2
- ✓ Decreased cocaine-induced locomotor sensitization

Conclusions

Effects of cocaine exposure are

not limited to the individuals exposed to cocaine*, but can*

be transmitted to their descendents in subsequent generations even though they were not exposed to cocaine.

Second-generation family members (or grandchildren) of parents who abused cocaine can show *biochemical, molecular, and behavioral* changes in the brain during development and at adulthood.

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