Incubation of cocaine craving: behavioral and neuronal mechanisms

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Special thanks:

The Waletzky family
SfN committee

Jane Stewart
Roy Wise

Lin Lu
Bruce Hope
Ongoing research projects

1. Stress-induced reinstatement of drug and palatable food seeking
   (Sunila Nair, Udi Ghitza)

2. Context-induced reinstatement of heroin seeking
   (Jennifer Bossert)

3. Developing a new model to assess relapse to cocaine and food seeking
   (with Abraham Zangen, Weizmann Institute, Israel)

4. Time-dependent increases in cocaine seeking after withdrawal
   (incubation of cocaine craving)
Outline

1. The clinical problem
2. Behavioral studies
3. Neurobiological studies
4. Conclusions and a brief summary of recent results on incubation of craving
5. Acknowledgements
The clinical problem: Relapse to cocaine use after prolonged abstinence

This relapse can be provoked by re-exposure to cocaine-associated cues that also induce drug craving.

Based on anecdotal evidence, Gawin and Kleber (1986) suggested that cue-induced cocaine craving increases over the first several weeks of abstinence and remains high over extended abstinence periods.

Research question: how do we study this “incubation” phenomenon in the laboratory rat?
An experimental procedure to study time-dependent changes in cue-induced cocaine seeking

**Cocaine self-administration:** 10 days

**Withdrawal (abstinence) period:** 1 to 180 days

↑ Extinction tests for cocaine seeking in a drug-free state
Time-dependent increases in cue-induced cocaine seeking after withdrawal from drug self-administration

Cocaine self-administration (10 days)

Withdrawal period (1 to 180 days)

Extinction tests for cocaine seeking in a drug-free state

60 days in rats ~ 5-6 years in humans

180 days in rats ~ 15-18 years in humans

Generality of the incubation phenomenon to other drug and non-drug rewards

Reward self-administration (10 days)  Withdrawal period (1 to 180 days)

Extinction tests

Heroin  Methamphetamine  Sucrose

Shalev et al. Psychopharmacology, 2001
Lu et al. Neuropharmacology, 2004
Shepard et al. Biol Psychiatry, 2004
More evidence for incubation of reward craving

**Cocaine 1**
Neisewander et al. 2000

![Graph showing lever presses over withdrawal days for Cocaine 1 experiment.]

**Cocaine 2**
Conard, Marinelli, Wolf, 2005

![Graph showing nose pokes over withdrawal days for Cocaine 2 experiment.]

**Cocaine 3**
Sorge and Stewart, 2005

![Graph showing lever presses over withdrawal days for Cocaine 3 experiment.]

**Alcohol**
Bienkowski et al. 2004

![Graph showing lever presses over withdrawal days for Alcohol experiment.]

**Heroin**
Di Ciano and Everitt 2004

![Graph showing lever presses over withdrawal days for Heroin experiment.]

**Sucrose**
Di Ciano and Everitt 2004

![Graph showing lever presses over withdrawal days for Sucrose experiment.]

* denotes significance.
Summary of behavioral findings

Incubation of reward craving:

- Long-lasting, but not permanent
- Observed with several drugs of abuse
- Observed with non-drug reinforcers
- Not evident after acute re-exposure to cocaine priming injections

The neuronal mechanisms of incubation of craving
Initial molecular findings

The neuroadaptation hypothesis of addiction

Chronic drug exposure causes long-lasting molecular, cellular, and neurochemical adaptations in the mesocorticolimbic dopamine system that underlie compulsive drug use and prolonged relapse vulnerability during abstinence

(Karler et al., 1989; Wolf and Khansa, 1991; Nestler et al. 1990; Kalivas and Stewart 1991)

The mesocorticolimbic dopamine system

Summary of initial molecular findings

Incubation of cocaine craving:

Associated with increases in peptide levels of brain-derived neurotrophic factor (BDNF) in VTA, accumbens and amygdala

Not associated with increases in protein levels of AMPA and NMDA glutamate receptor subunits in VTA, accumbens and amygdala

Not associated with increases in protein levels of cyclin-dependent kinase 5 (cdk5) and tyrosine hydroxylase (TH) in VTA and accumbens

Not associated with increases in the activity of cAMP-dependent protein kinase (PKA) and adenylate cyclase (AC) in VTA and accumbens

Grimm et al. J Neurosci 2003
Lu et al. J Neurochem 2003, 2005
Lu et al. J Neurosci 2004
Role of central amygdala ERK in incubation of cocaine craving

Lin Lu

Collaborator: Bruce Hope

A key regulator of synaptic plasticity and learning and memory (Sweatt 2001)

Amygdala ERK is involved in conditioned fear responses (Schaffe et al. 2000)

Mesolimbic ERK is activated by cocaine (Licata and Pierce 2003; Valjent et al. 2000)

The human amygdala is activated by cocaine cues (Grant et al. 1996; Childress et al. 1999)

ERK (extracellular signal-regulated kinase)

From Thomas and Huganir. Nature Neuroscience, 2004
Behavioral data: Training and extinction tests

Cocaine/saline self-administration training (10 days)  
Withdrawal period (1 or 30 Days)  
Extinction test Day 1  
Extinction test Day 30

Training

Infusions (6 h)

Cocaine (n=34)

Saline (n=34)

Training day

Extinction test

Lever presses (30 min)

Saline-trained rats  
Cocaine-trained rats

Lu, Hope et al. *Nature Neuroscience*, 2005
Exposure to cocaine cues increases ERK phosphorylation in the **central amygdala** after **30 days** of withdrawal.
Inhibition of ERK phosphorylation in the central amygdala attenuates cocaine seeking after 30 days of withdrawal.
Inhibition of ERK phosphorylation in the **basolateral amygdala** has **no effect** on cocaine seeking after **30 days** of withdrawal.
Induction of ERK phosphorylation in the central amygdala restores cocaine seeking after 1 day of withdrawal.

**Phosphorylated ERK**

<table>
<thead>
<tr>
<th>% of vehicle values</th>
<th>Vehicle</th>
<th>NMDA (25 ng)</th>
<th>NMDA (250 ng)</th>
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<tbody>
<tr>
<td>Central amygdala</td>
<td><img src="chart" alt="" /></td>
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<tr>
<td>Basolateral amygdala</td>
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**Extinction responding**

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<th>Lever presses (30 min)</th>
<th>NMDA dose (ng/site)</th>
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<tr>
<td></td>
<td>0</td>
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NMDA receptor

NMDA

CeA

Induction of ERK phosphorylation in the central amygdala restores cocaine seeking after 1 day of withdrawal.
Blockade of NMDA receptors in the central amygdala attenuates cocaine seeking after 30 days of withdrawal.

**Phosphorylated ERK**

![Graph showing phosphorylated ERK levels in the central and basolateral amygdala.](image)

**Extinction responding**

![Graph showing extinction responding in vehicle and AP-5 conditions.](image)

**Diagram**

![Diagram illustrating the blockade of NMDA receptors and their effects on ERK and MEK pathways.](image)
More evidence for a role of central amygdala glutamate in incubation of craving

LY379268: a selective agonist of mGluR$_{2/3}$ receptors that decreases evoked glutamate release

- **Day 21 withdrawal**
- **Day 3 withdrawal**

**Systemic injections**

<table>
<thead>
<tr>
<th>LY379268 dose (mg/kg, i.p.)</th>
<th>Lever presses (3 h)</th>
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<tbody>
<tr>
<td>0</td>
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<tr>
<td>1.5</td>
<td>150</td>
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<td>3.0</td>
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**Central amygdala injections**

<table>
<thead>
<tr>
<th>LY379268 dose (µg/site)</th>
<th>Lever presses (3 h)</th>
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<tbody>
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**Basolateral amygdala injections**

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Lu et al. Biological Psychiatry 2006
Conclusions

Incubation of reward craving is a general phenomenon that occurs with both drug and non-drug rewards.

Time-dependent increases in the responsiveness of central amygdala ERK and glutamate to cocaine cues mediate the incubation of craving.

Implications for treatment

Drug addicts may be very vulnerable to relapse at time periods that are far beyond the acute drug withdrawal phase.

Time away from drug (e.g., incarceration) is not a good method for relapse prevention.

Our studies suggest that mGluR2/3 agonists should be considered in the treatment of relapse to cocaine and other drugs.
Exposure to cocaine cues increases ERK phosphorylation in the medial prefrontal cortex after 30 days of withdrawal.

An update on incubation of cocaine craving (1)

Koya, Uejima et al. SFN 2006
An update on incubation of cocaine craving (2)

Incubation of cocaine craving:

- Enhanced in GluR1 knockout mice (Mead et al. Neuropsychopharmacol. 2006)
- Attenuated by inhibition of protein synthesis in the amygdala following cue exposure during early withdrawal (Lee et al. J. Neurosci. 2006)
- Associated with increases in neuronal activity in the accumbens (Hollander & Carelli. Neuropsychopharmacol. 2006)
- Associated with greater total production and surface expression of GluR1 in the accumbens (Conrad et al. SFN 2006)

Incubation of cocaine craving in humans:

Incubation of cocaine relapse during a disulfiram clinical trial (Kosten et al. CPDD 2005).

“In support of human ‘incubation’, only 3% of cocaine dependent subjects who stopped cocaine use for at least 2 weeks relapsed before week 4, and relapse peaked after 6.6 weeks of abstinence”
Acknowledgments

• Present post-doctoral fellows: Jennifer Bossert, Sunila Nair

• Present students: Jamie Uejima, Gabriela Poles, Sam Golden

• Former post-doctoral fellows: Yvona Buczek, David Highfield, Jeff Grimm, Hans Crombag, Uri Shalev, Lin Lu

• Former students: Shirley Leung, Alison Clements, Jasmine Yap, Kelly Badger, Polly Robarts, Shirley Liu, Jack Dempsey, David Chuang, Robert Busch, Deepti Nagarkar, Sean Sheffler-Collins, Sarah Gray

• Intramural collaborators: Bruce Hope, Marisela Morales, Eisuke Koya, Udi Ghitza, Jack Shepard, Tsung-Ping Su, Teruo Hayashi, Brandon Harvey, Michael Baumann, George Uhl, Tim Liu, Kenzie Preston, David Epstein, Zhi-Bing You, Roy Wise, Barry Hoffer

• Extramural collaborators: Jane Stewart, AD Le, Doug Funk, Geoff Schoenbaum, Taco de Vries, Paul Fletcher, Diane Lattemann, Gal Yadid, Abraham Zangen, Suzanne Erb, Micky Marinelli

• Final thanks: Pier-Vincenzo Piazza, Barry Everitt, Neil Grunberg, Klaus Miczek