LMO and ALK
The Unbelievable Likeness of Beings

Heberlein Lab
UCSF and EGCRC
Why flies ???

Because we believe:
- similarity of drug-induced behaviors
- conservation of synaptic machinery involved in drug responses
- conservation of genetic bases of drug-related behaviors

Because we can:
- awesome classical and molecular genetics
- economy of scale
- robust behaviors
- sophisticated tools to manipulate genome and neural circuits
The Approach

1. Develop behavioral model
2. Genetic screens
3. Mutants
4. Genes
   - Candidate genes
   - Mechanisms
5. Targets for pharmacotherapy
Acute cocaine exposure

McClung and Hirsh

100 µg cocaine

1st min

2nd min

3rd min

4th min

Linus Tsai
Mock exposure

McClung and Hirsh

Linus Tsai
A simple behavioral assay

Cocaine / Nicotine Exposure

“Crackometer”

\[
\frac{(2\times5) + (1\times1) + (0\times2)}{2\times \text{total # flies}} \times 100 = \text{BS, %}
\]

Bainton, Tsai et al., Current Biology, 2000
## Cocaine Sensitivity Mutants

(of ~ 400 lines screened)

<table>
<thead>
<tr>
<th>Line</th>
<th>Phenotype</th>
<th>Gene or homology</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP1a</td>
<td>RRR</td>
<td>RhoGAP18B (<em>white rabbit</em>)</td>
</tr>
<tr>
<td>EP1b</td>
<td>RRR</td>
<td>RhoGAP18B (white rabbit)</td>
</tr>
<tr>
<td>EP1c</td>
<td>RR</td>
<td>Alpha-mannosidase</td>
</tr>
<tr>
<td>EP2</td>
<td>RRR</td>
<td>MAPKKK (<em>dtao</em>)</td>
</tr>
<tr>
<td>EP3</td>
<td>RR</td>
<td>MAPKKK (dtao)</td>
</tr>
<tr>
<td>EP4a</td>
<td>S</td>
<td>Bx / LMO</td>
</tr>
<tr>
<td>EP4b</td>
<td>SS</td>
<td>Bx / LMO</td>
</tr>
<tr>
<td>EP5</td>
<td>SS</td>
<td>GPCR (<em>moody</em>)</td>
</tr>
<tr>
<td>EP6</td>
<td>SS</td>
<td>GSK3 (<em>shaggy</em>)</td>
</tr>
<tr>
<td>EP7</td>
<td>SS</td>
<td>mRNA binding protein</td>
</tr>
<tr>
<td>EP8</td>
<td>S</td>
<td>Neuropeptide (<em>amnesiac</em>)</td>
</tr>
<tr>
<td>EP9</td>
<td>S</td>
<td><em>period</em></td>
</tr>
<tr>
<td>EP10</td>
<td>S</td>
<td>Ethanolamine kinase (<em>easily shocked</em>)</td>
</tr>
</tbody>
</table>

Linus Tsai, Roland Bainton
Effect of *dlmo* mutations on cocaine sensitivity

**Loss-of-function**


- Drug Effect Score, %
  - Ctl-1: *
  - EP1383: *
  - EP1306: *
  - Ctrl-2: *
  - pdrm: *
  - Ctrl-3: *

**Gain-of-function**

- Genotype: Ctl, l, B, x, B, x

- Drug Effect Score, %
  - Ctl: *
  - l: *
  - B: *
  - x: *

*dlmo* expression in NS

Tsai et al. PLOS, 2004
Cocaine sensitivity is inversely proportional to [dLMO]
How does dLMO work?

Milan and Cohen, 1999
Rincon-Limas et al., 2000
ALK: A Receptor Tyrosine Kinase

ALK Pathway

- Oncogenic when fused to other proteins through chrom. translocations (Morris et al, 1994)

- Activation of Alk promotes neurite outgrowth in culture (Souttou et al, 2001)

- Expressed primarily in brain, particularly during development (Iwahara et al, 1997; Vernersson et al, 2006)

- KO mice viable, exhibit “antidepressant” phenotype (Bilsland et al, 2008)
Drug sensitivity

- dlmo
- dAlk

Cocaine sensitivity

- lof
- wild type
- gof
dlmo → dAlk

cocaine sensitivity
Mammalian LMOs interact with multiple partners

LMOs can act as repressors or activators of transcription complexes
A null allele of *Lmo4* generated from gene-trap ES cells

http://baygenomics.ucsf.edu/

Heterozygous mice show enhanced cocaine sensitivity

Lasek et al., 2010
LMO4-GAL fusion protein expression in adult \( Lmo4^{gt/+} \) mouse brain

Lašek et al., 2010
Brain regions implicated in addiction-related behaviors

Nestler, 2001
Where and when is Lmo4 function required to regulate cocaine-related behaviors?

Virally-mediated RNA interference
Viral delivery to and infection of Nucleus Accumbens

Lasek et al., 2010
shRNA reduces *Lmo4* mRNA and protein in Acb

Lasek et al., 2010
Sensitization: A long-lasting neuroadaptation induced by repeated drug exposure

learning and memory
“liking” ------ “wanting” ------ “craving”
Cocaine sensitization enhanced by *Lmo4* RNAi in Acb

![Graph showing distance traveled over days with different treatment groups.](image)

**Legend:**
- Green square: Lmo4.3 cocaine
- Green circle: Lmo4.3 saline
- Purple square: SCR cocaine
- Purple circle: SCR saline

**n = 9**

*Lmo4* function in the adult Acb to regulate the acquisition and maintenance of cocaine sensitization

*Lasek et al.*, 2010

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**Note:**
- Distance traveled, cm $\times 10^{-3}$
- Day 1 (saline), Day 2 (saline), Day 3, Day 4, Day 5, Day 6, Day 7, Day 17 (cocaine)
Does $Lmo4$ inhibit $Alk$ expression in Acb?
Does LMO4 regulate \textit{Alk} expression directly?

LMO4 is associated with \textit{Alk} promoter in striatum, which is expected to inhibit \textit{Alk} expression.

Amy Lasek (unpublished)
Is *Alk* involved in cocaine sensitization?

**Enhanced sensitization**

**Prediction:** Down-regulation of *Alk* should reduce/impair cocaine sensitization
Alk downregulation in Acb...

... reduces rate of acquisition of cocaine sensitization

Amy Lasek (unpublished)
LMO4-ALK... does it matter?

Cocaine sensitization

Acute inhibition of ALK delays cocaine sensitization and reduces the rewarding properties of cocaine.

ALK inhibitors are in development for cancer, ALK may therefore be a novel therapeutic target for addiction.

Amy Lasek (unpublished)
Not just ALK..... but its signaling pathways!

ALK Pathway

Pharmacotherapy available / in development
Not just ALK..... but its signaling pathways!

Pharmacotherapy available / in development
Validation of \textit{Lmo4} in cocaine sensitivity and sensitization

\textit{Alk} regulated by \textit{LMO4}

\textit{dAlk} RTK regulated by \textit{dLMO}

\textit{dLmo} mutants

Cocaine sensitivity screen

\textit{Alk} regulates cocaine sensitization

ALK small-molecule inhibitor reduces cocaine sensitization and reward
The mouse team (EGCRC)

Amy Lasek  Francesco Giorgetti
David Kapfhamer  Julie Gesch
How does LMO4 regulate Alk expression??

Poster by Amy Lasek
Marco Milan, Steve Cohen, Nick Justice, Yuh-Nung Jan

Steve Morris (St. Jude’s)
EGCRC Animal Core Facility
EGCRC Histology Core Facility

NIH/NIDA
State of California
Department of Defense
Thank you!