Modeling behavioral endophenotypes related to alcohol abuse in mice

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What can rodent models do to enhance the studies of alcohol abuse and alcoholism?

- Animal models can provide one strategy to study traits that predate the disorder or are associated with the disease including

  **Broad Categories of Endophenotypes:**
  behavioral, cognitive, neurophysiological, or neurochemical processes that are associated with risk for alcohol abuse

- Provide multiple different strategies to identify candidate genes regulating these phenotypes.
Goal of Using Endophenotypes for Dissection of Complex Disorders

Decreased complexity of both phenotype and genetic analysis

Example: working memory impairments in schizophrenia

Increased complexity of both phenotype and genetic analyses

Less # of Genes

More

Adapted from Figure 1: Gottesman, I.I. and Gould, T.D.: Amer. J. Psychiatry 2003; 160: 636-645
All Behavioral Traits are Regulated by Multigenic or Polygenic Systems

Modeling of Phenotypes related to the predisposition to alcoholism and assessing the actions of alcohol

Example 1: The role of $\gamma$-Protein Kinase C
- Initial sensitivity---Low Responding
- Anxiety and risk taking
- Behavioral Disinhibition
- Ethanol consumption

Example 2: The role of nicotinic cholinergic receptors in mediating alcohol/nicotine interactions
- Startle
Genetic Strategies to Study Complex Behaviors

Single gene
- Essential genes for Behaviors, Physiology etc.
  - Transgenics and Null Mutants

Polygenic
- Genes regulating variation in humans and animals
  - Strain Differences
  - Recombinant Inbreds & QTL
  - Selected lines
Sensitivity, Anxiety, Risk taking, Behavioral Disinhibition

What genes regulating these pharmacological and behavioral traits?
Protein Kinase C is a Central Regulator of Diverse Pathways in the Brain

PKC

AC

Ca/CAM BINDING PROTEINS

NEUROGRANIN

GABA

5HT

DA

MUSCARINIC

5HT

DA

LIGAND-GATED CHANNELS

GABA

5HT

R

G

PIP

DAG

IP$_3$

Ca$^{++}$

ER

PKC

Ca$^{++}$

G$\gamma$
PKC Super Gene Family

PKC-α

PKC-β

PKC-γ

PKC-η

PKC-ε

PKC-δ

PKC-θ

PKC-ι

PKC-ζ

PRK1

PRK2

Neuronal Expression
Post natal Expression
Postsynaptic localization
γ-PKC Knock-out Mice:

• Created using ES cell technology

• Deletion inserted in γ-PKC gene

• Lack expression of γ-PKC protein throughout brain BUT especially important in cerebellum, hippocampus, striatum, and amygdala

• Mild hind limb ataxia in mutants
Sensitivity

\( \gamma - \text{PKC} \)
Sensitivity

Low response associated with increased risk for alcoholism: ataxia and other subjective measures (Schuckit et al.)
Increased sensitivity associated with lower risk (Heath et al.)

Confounds in Human Studies:

1. History of alcohol exposure and smoking (Madden, Heath, Martin)
2. Role of Acute Functional Tolerance

In our animal studies:

Can control #1 but #2 is more difficult
Sensitivity to High Doses of Ethanol

- Mutants are less sensitive to first exposure to ethanol
- Ethanol Clearance was not different

3.5 g/kg I.P

<table>
<thead>
<tr>
<th>MUT</th>
<th>HET</th>
<th>WT</th>
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<tr>
<td>285.3 + 14.8 mg%</td>
<td>224.9 + 19.9 mg%</td>
<td>230.3 + 44.7 mg%</td>
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What neurotransmitter system could be altered due to loss of $\gamma$-PKC?

Alterations in GABAergic system

- Reduced ethanol-potentiation of Muscimol-stimulated chloride flux in microsacs from cerebellum, midbrain, and cortex

Harris/Wehner Collaboration (PNAS 92: 3658-3662, 1995)

Additional Questions???

- Is there an electrophysiological correlate to this?
- Is $\gamma$-PKC the only PKC isotype involved?
PKC Super Gene Family

PKC-α

PKC-β

PKC-γ

PKC-δ

PKC-θ

PKC-η

PKC-ε

Neuronal Expression
Post natal Expression
Postsynaptic localization

Expressed in many tissues
Shown to change with chronic treatment in PC12 cells

PRK1
PRK2
Proctor et al. JPET 305: 264-270, 2003

ε− PKC null mutant mice:
• more sensitive to ethanol compared to wild types
• will self-administer less ethanol (Hodge et al.)
Ethanol differentially modulates GABA<sub>\text{A}</sub> IPSC responses in PKC<sub>\text{Y}</sub> and PKC<sub>\text{E}</sub> wild type and knockout mice

Hippocampal Recordings
Correlation of ethanol modulation on GABA\textsubscript{A} responses and duration of loss of righting reflex in PKC mouse lines

![Graph showing correlation between GABA\textsubscript{A} IPSC response (% of control) and ethanol sleep time (min; 3.5 g/kg). The graph includes data points for PKC\textsubscript{\gamma}\textsuperscript{++}, PKC\textsubscript{\gamma}\textsuperscript{+/−}, PKC\textsubscript{δ}\textsuperscript{++}, and PKC\textsubscript{δ}\textsuperscript{−/−} with the correlation coefficient r\textsuperscript{2} = 0.95.]
We conclude: $\gamma$PKC and $\varepsilon$PKC isotypes may be important regulators of initial sensitivity for systems that may involve GABAergic function.

**BUT** initial sensitivity is not one precise phenotype.

Are low dose behavioral effects different between mutants and wild types?

- $\gamma$-PKC mutants are also less sensitive to low-dose effects.
γPKC

- Sensitivity
  - Mutation leads to reduced sensitivity

- Anxiety, Risk taking
  - Note: Novelty-seeking has been hard to model
PKCγ null mutants may be risk takers...

Ha! I've outwitted them at last!

Slide from Jason Keller, Wehner lab
Elevated Plus Maze

Mirrored Chamber Test

Open Field Arena
Mutants demonstrate less anxiety or greater exploration of novel places.
\(\gamma\)PKC mutants appear less anxious and again are willing to explore novel places.
Open-field Studies

Security is a nice wall to hug!

That eagle will never get me. I am invincible!!!
Open-field behavior under white light in γ-PKC mice

Mutants are more willing to explore center and spend more time there consistent with increased risk taking or less anxiety.
Mutation leads to reduced sensitivity

Mutation leads to reduced anxiety or increased Risk taking

Mutation leads to reduced sensitivity

Mutation leads to reduced anxiety or increased Risk taking
Human Genetic Modeling of Behavioral Disinhibition

Behavioral Disinhibition

- Experimentation is driven by environmental factors
- Severe Substance Abuse with early onset has a large genetic component
- Colorado Adolescent Drug Dependence Research Center

From Young et al:
Measuring impulsivity in the mouse

- Appetitive learning using an operant paradigm

Slide from Dr. Barbara Bowers
SIGNALED APPETITIVE TASK

DRL task: differential reinforcement of low rate of responding

1. Mice deprived to 85% of normal weight
2. Mice learn to nose poke for a food reward. (FR 1, FR 3)
3. Mice learn to associate reward with the presentation of a clicker sound.
4. Mice must learn to withhold their nose-poking response until tone to gain a reward on a variable schedule. Clock is reset when nose poke is not appropriate response.
Inbred Strain survey provides first evidence for genetic regulation of the withholding response.
Impulsivity is negatively correlated with Ethanol consumption.

$r = -0.63; \ P < 0.05$
γ-PKC Null mutants are impaired on withholding responses to receive the sucrose reward

- What neurotransmitter system mediates this response?
  5HT 2a/c receptors?? - Bowers

Sensitivity

Anxiety Risk taking

Behavioral disinhibition

Increased alcohol consumption
γ-PKC mutants consume more ethanol in a free-choice 2 bottle choice test.
There is no difference in saccharin and nicotine preference or consumption based on genotype.
Increased alcohol consumption

**γPKC**

- Sensitivity
  - Mutation leads to reduced sensitivity
- Anxiety, Risk taking
  - Mutation leads to reduced anxiety or increased risk taking
- Behavioral Disinhibition
  - Mutation leads to increased impulsivity

Increased alcohol consumption
Conclusions about $\gamma$-PKC

$\gamma$-PKC mutation has pleiotropic effects on phenotypes that may predispose individuals to greater risk of alcohol abuse

Translating these results to humans

- Are there human polymorphisms in the $\gamma$-PKC gene?
- Are they associated with any measures of risk for alcoholism or drug abuse?
Gene structure of PRKCG: Location of SNPs Selected

Drs. Marissa Ehringer
- SNP association analyses on subjects from Colorado Adolescent Drug Dependence Center
Example 2:

- Collaborative work with Allan Collins, IBG
- The role of nicotinic receptors in mediating sensitivity to ethanol’s effects the startle response
• Most alcoholics are heavy smokers

• Common genes may influence sensitivity to nicotine and ethanol

• Startle response is a simple behavior that is altered by both ethanol and nicotine

• FH+ and FH- individuals differ in basal acoustic startle and after ethanol consumption

• Ethanol can modulate function of $\alpha_4\beta_2$ nAChRs \textit{in vitro}
A. Alpha 2, 3, 4, 5, 6, 7..9,10 Beta 2, 3, 4

B. A4β2* highly expressed in Brain
In Situ Hybridization for nAChR Subunits from Michael Marks, CU

Sections approximately -1.8 mm Bregma
ACOUSTIC STARTLE

• Acoustic startle measured at 100-120 dB
• Dose-response analyses for effects of nicotine and ethanol

Drawing from Dr. Karen Stevens
Multiple strategies to provide converging evidence

1. Long Sleep/Short Sleep mice
2. LS X SS Recombinant inbred strains
3. Nicotinic receptor mutants
α4 Missense Mutation in LS X SS RI STRAINS

• LS and SS RI strains have a polymorphism at position 529
  LS-like = Threonine
  SS-like = Alanine
• Confers a change in receptor function

From: Dr. Jerry Stitzel, Institute for Behavioral Genetics
Creation of LS × SS Recombinant Inbred (RI) Strains

strain 1 (LS) \( \times \) strain 2 (SS)

\( F_1 \) \( \times \) \( F_2 \)

20 generations of brother-sister matings

RI Strains
Results in LSXSS Recombinant Inbreds

- Strains containing the T529 variant were less sensitive to the effects of ethanol on acoustic startle.

- A/T polymorphism accounted for 56% of variation.

- Tritto et al. (2002) showed same relationship for nicotine’s effects startle

- Suggests a role for $\alpha$4-containing receptors in mediating the effects of ethanol on startle

- Animal models were needed to test this role of $\alpha$4-containing receptors more directly.
Gain of Function Mutation in $\alpha 4$ Nicotinic Subunit

- In brain usually in heteromer as $\alpha 4\beta 2$
  - Acetylcholine
  - Nicotine
- Do alcohol and nicotine have any common sites of action?

Leucine 9’ Serine Mutation: Gain of function mutation increases sensitivity to acetylcholine and nicotine

Labarca et al. PNAS: 98: 2786-2791, 2001
β2 Null Mutants

- Virtually all α-containing nAChRs include the β2 subunit.
- α4β2 receptors are eliminated in β2 null mutants.
- The β2 null mutants have reduced sensitivity to nicotine on multiple measures.

Prediction:
- Gain of function mutants should be MORE sensitive to ethanol
- Null mutants should be LESS sensitive to ethanol
Ethanol Effects on Startle in $\alpha 4$ and $\beta 2$ mice

- $\alpha 4$ L9'S Hets are more sensitive to the effects of ethanol

- $\beta 2$ mutants are less sensitive to the high-dose effects of ethanol
Conclusions and Future Studies

- $\alpha 4\beta 2$-containing receptors may play important roles in modulating the effects of ethanol and nicotine on acoustic startle response

- Evaluate the A529T $\alpha 4$ subunit polymorphism using a knock-in mouse line
  
  Drs. Gregg Homanics (PITT) and Jerry Stitzel (IBG)

Translating this to humans

Dr. Marissa Ehringer: examining nicotinic gene family
Dr. Kent Hutchinson: $\alpha 4$ with startle response
Alcoholism

New Animal Model with human SNP

Association studies

Human SNP analysis

Analyse phenotypes of interest in mice

Find genetic mouse models to suggest candidate genes
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<th>Contributors to the work</th>
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<td><strong>PKC WORK</strong></td>
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<td>Jason Keller</td>
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<td><strong>Nicotinic Work</strong></td>
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Funded by NIAAA