2004

Modeling behavioral endophenotypes related to alcohol abuse in mice

Jeanne M. Wehner
University of Colorado

Modeling Behavioral Endophenotypes Related to Alcohol Abuse in Mice

Jeanne M. Wehner
Institute for Behavioral Genetics
University of Colorado

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

Less — More

# of Genes

Increased complexity of both phenotype and genetic analyses

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

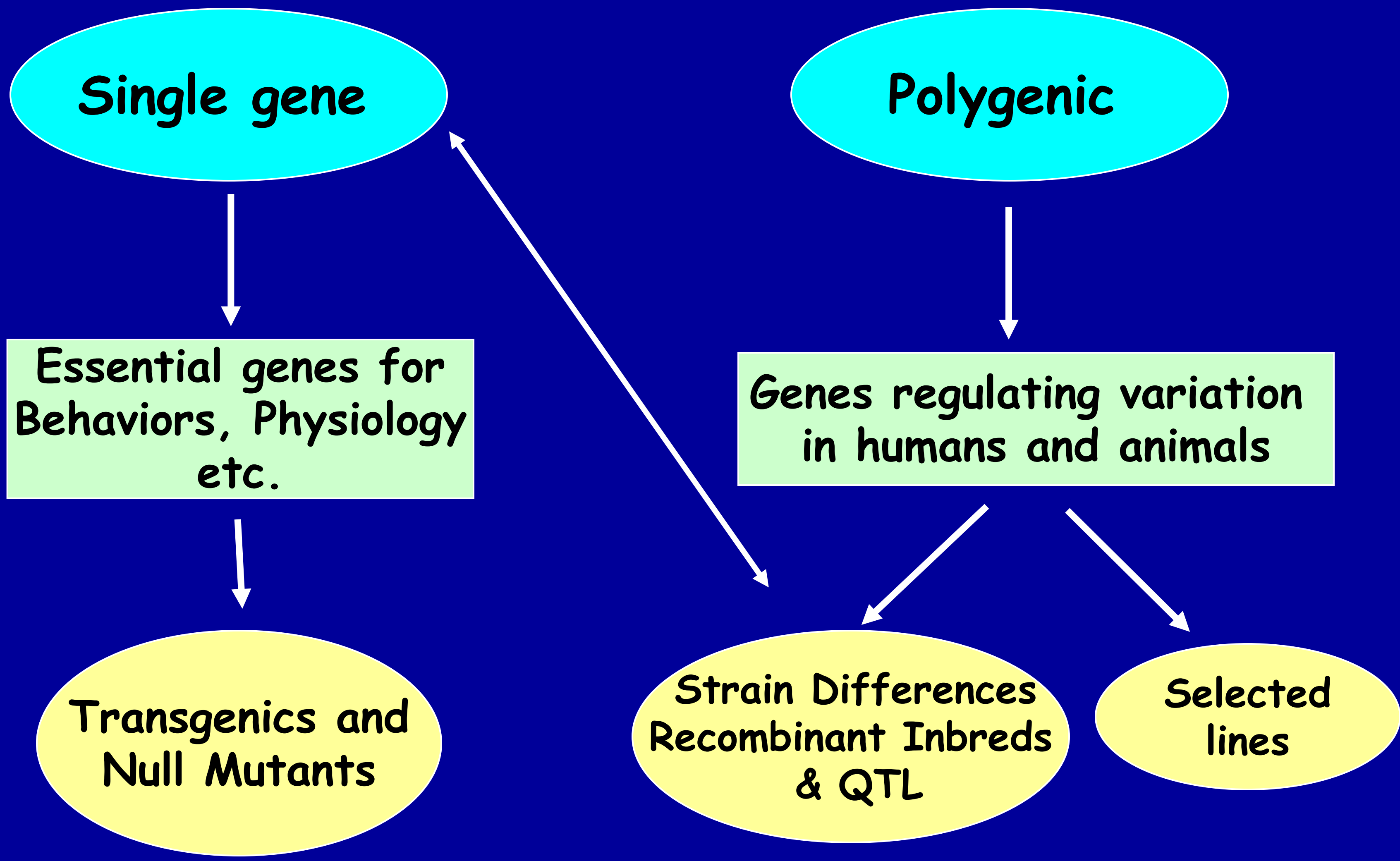
Example 1: The role of γ-Protein Kinase C

- 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

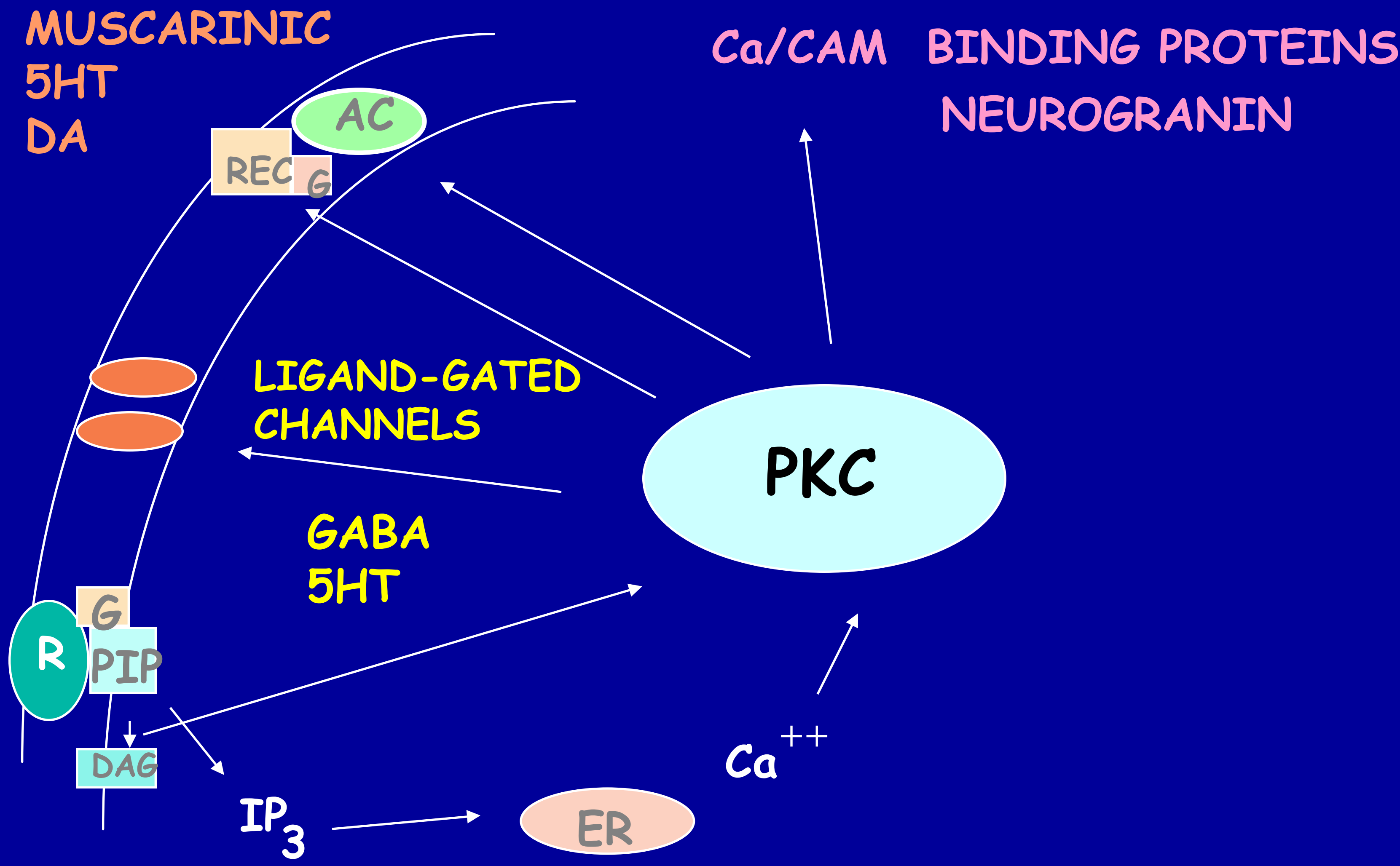


Example 1: Protein Kinase C

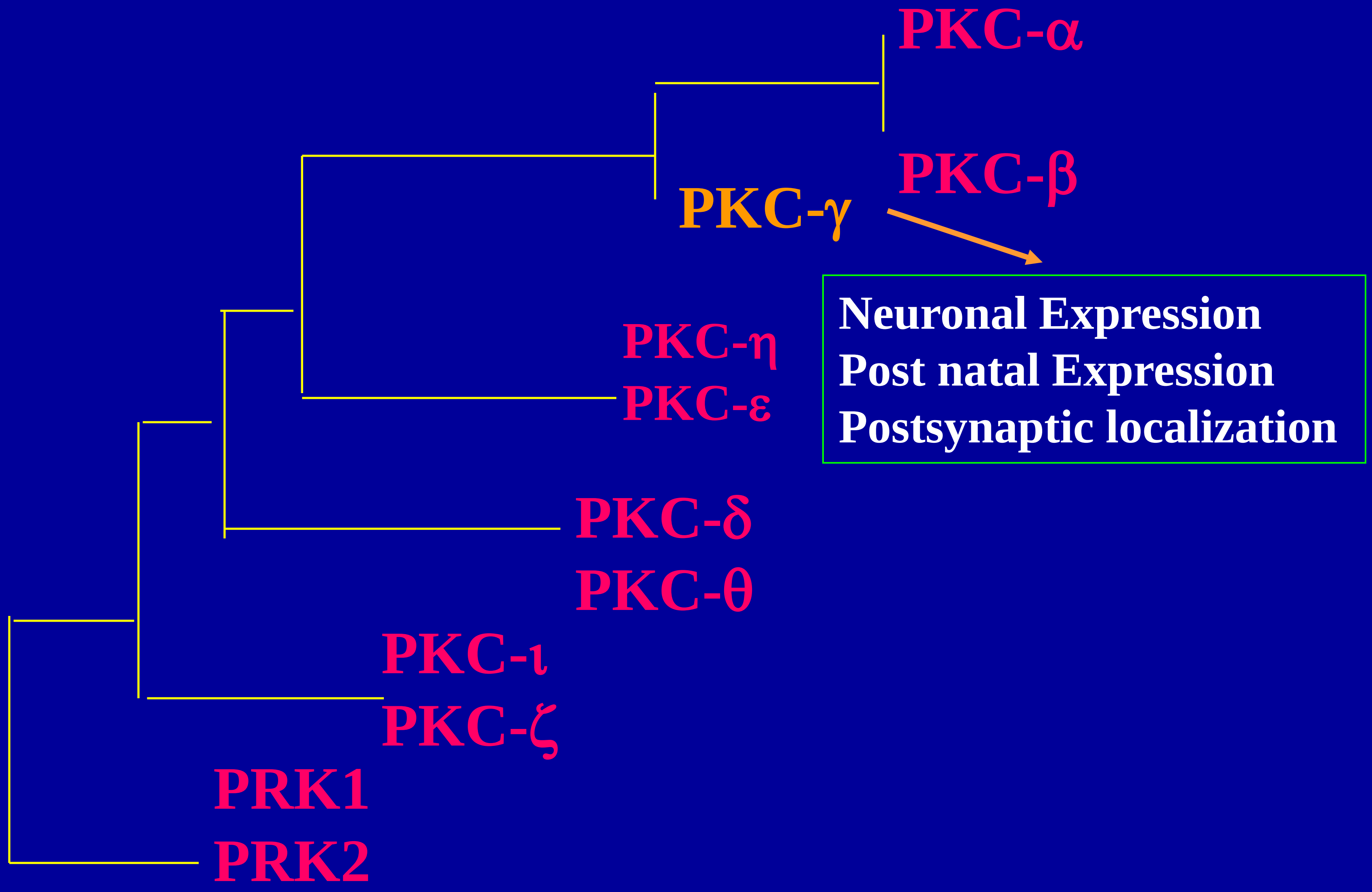
Modeling Possible Predisposing Factors

What genes regulating these pharmacological and behavioral traits ??

Protein Kinase C is a Central Regulator of Diverse Pathways in the Brain

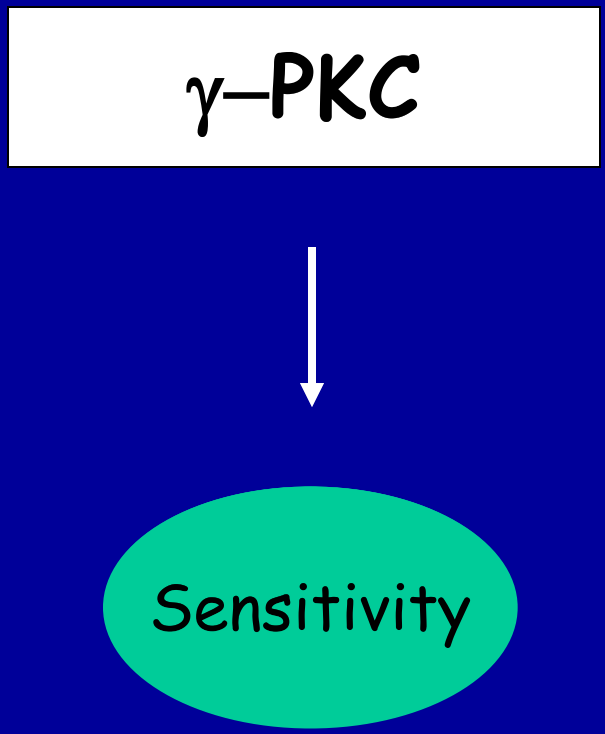


PKC Super Gene Family



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


γ–PKC
Sensitivity

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.)

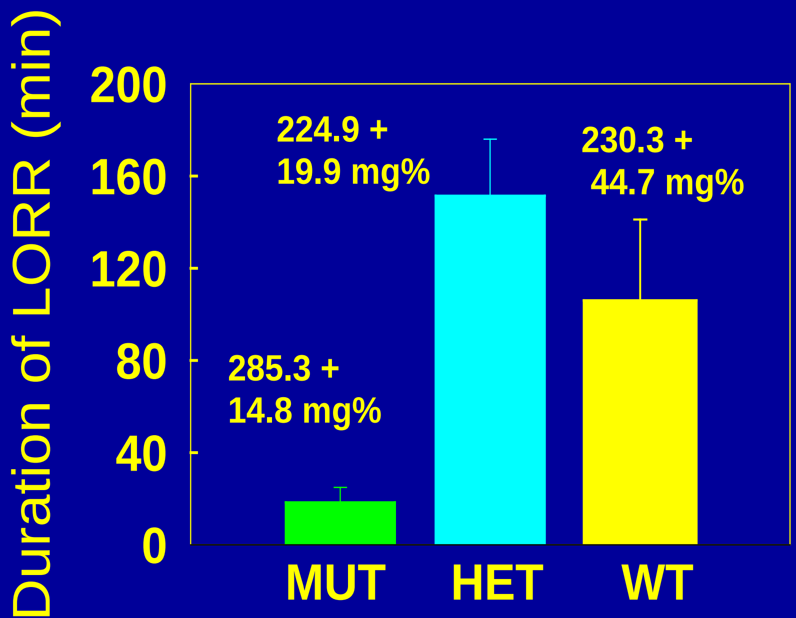
Confounds in Human Studies:

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



3.5 g/kg I.P

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

What neurotransmitter system could be altered due to loss of γ-PKC ?

Alterations in GABAergic system

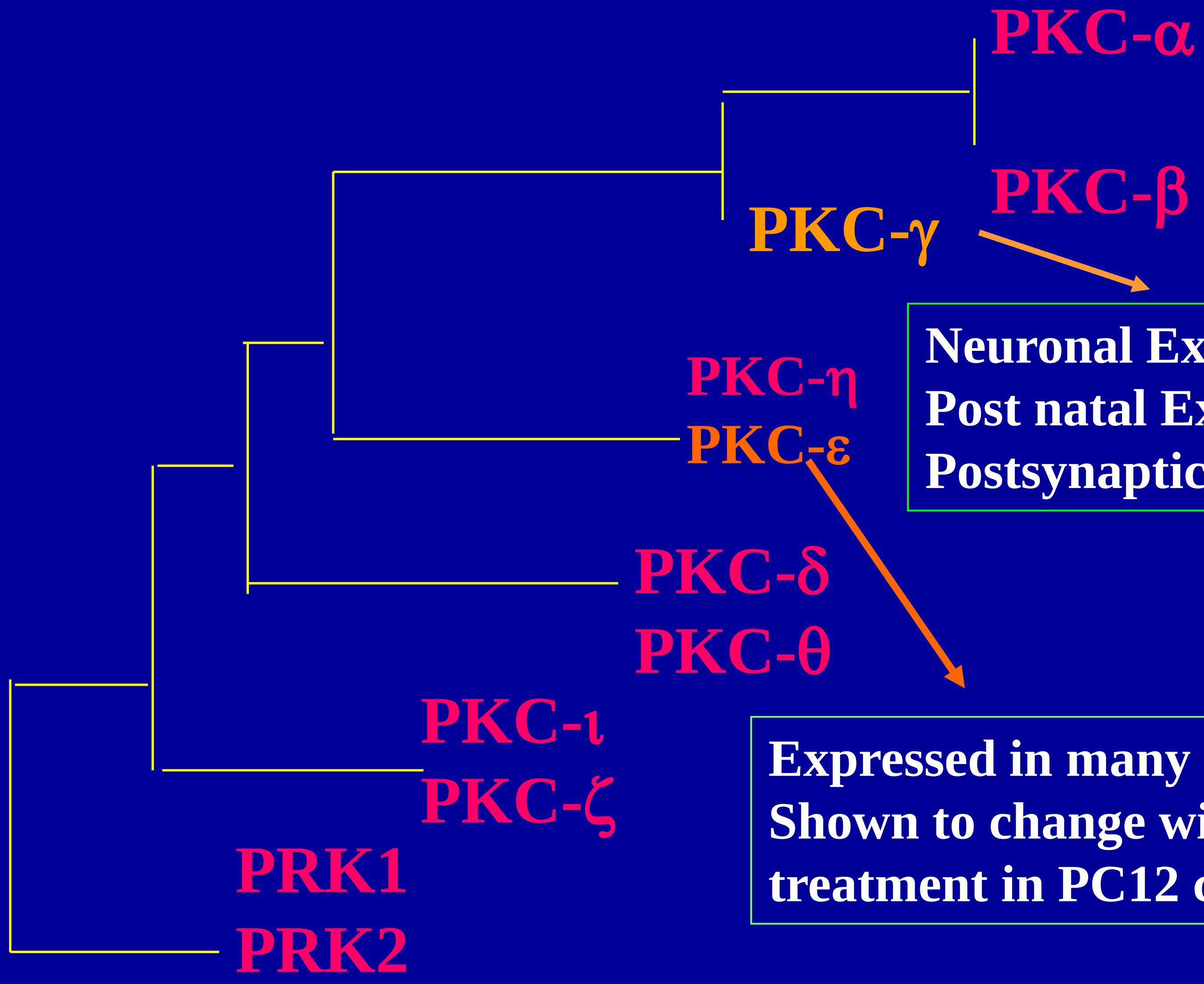
- Reduced ethanol-potentiation of Muscimol-stimulated chloride flux in microsacs from cerebellum, midbrain, and cortex

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

Additional Questions???

- Is there an electrophysiological correlate to this?
- Is γ-PKC the only PKC isotype involved?

PKC Super Gene Family

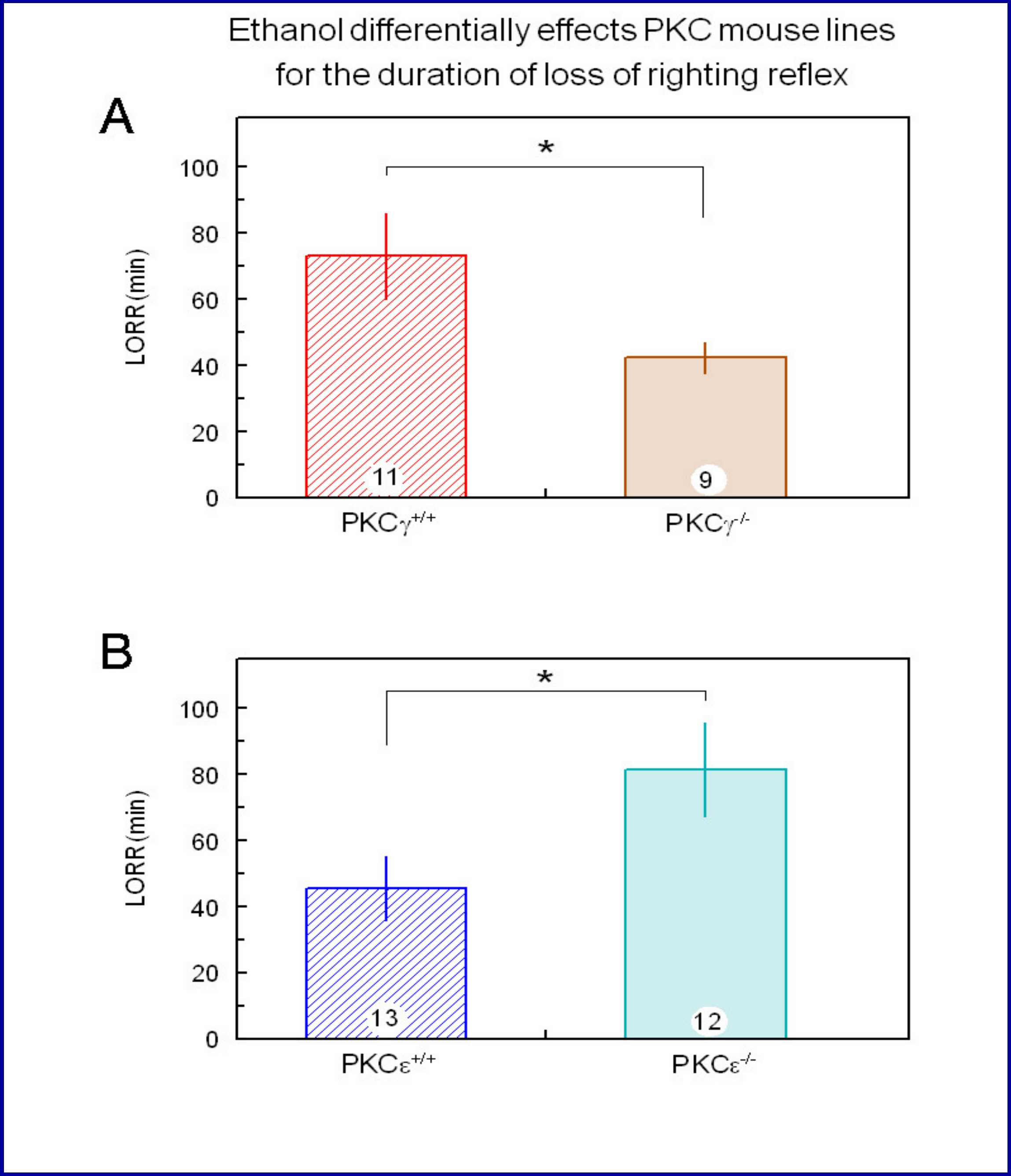


Neuronal Expression
Post natal Expression
Postsynaptic localization

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

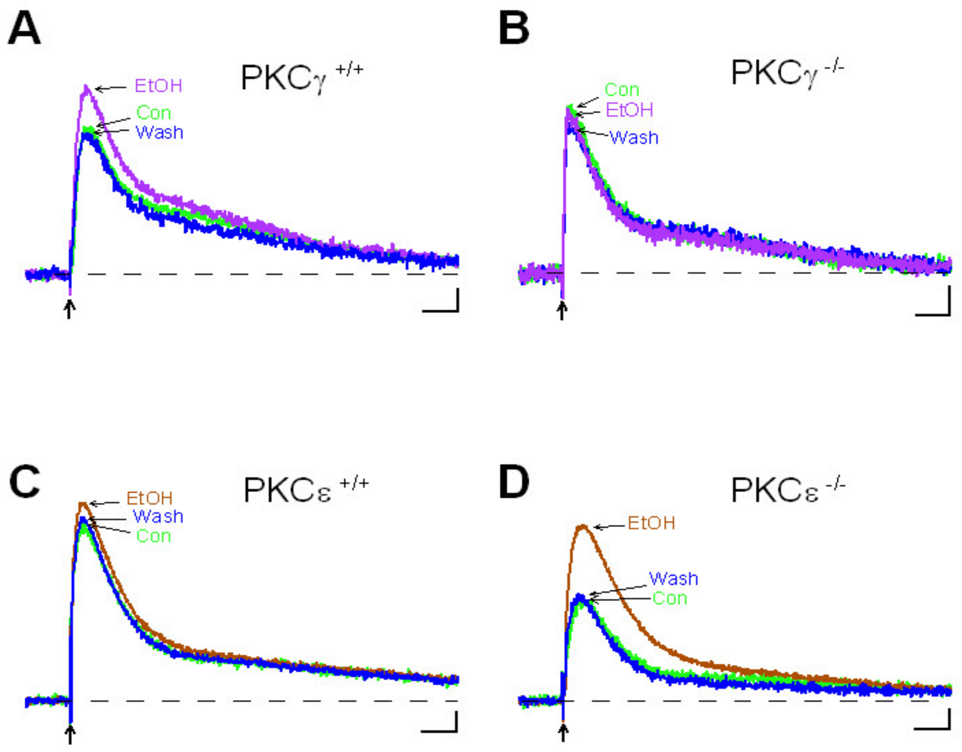
ε– PKC null mutant mice:

- *more* sensitive to ethanol compared to wild types
- will self-administer less ethanol (Hodge et al.)

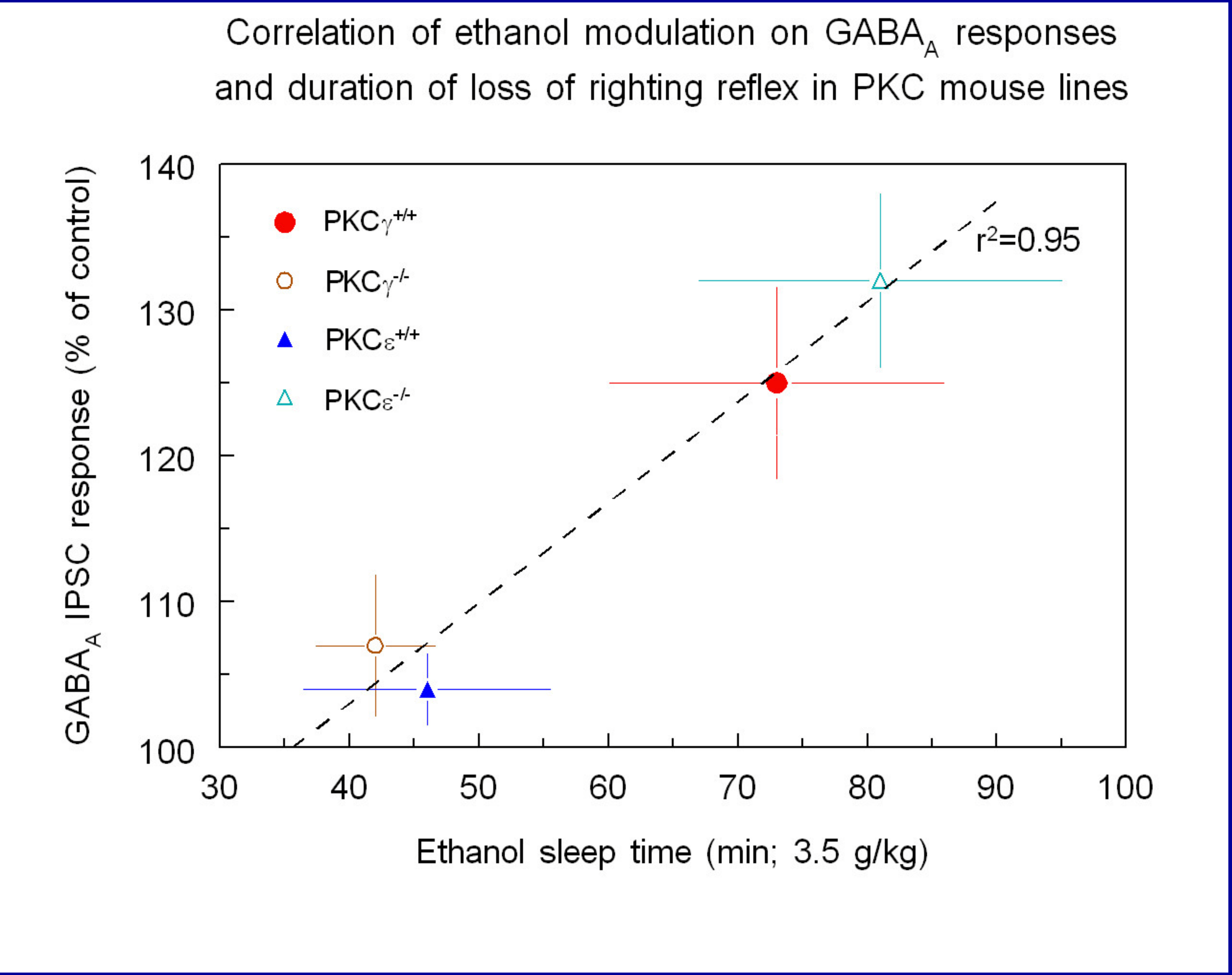


Proctor et al. JPET 305: 264-270, 2003

Ethanol differentially modulates $GABA_A$ IPSC responses in PKCγ and PKCε wild type and knockout mice



Hippocampal Recordings



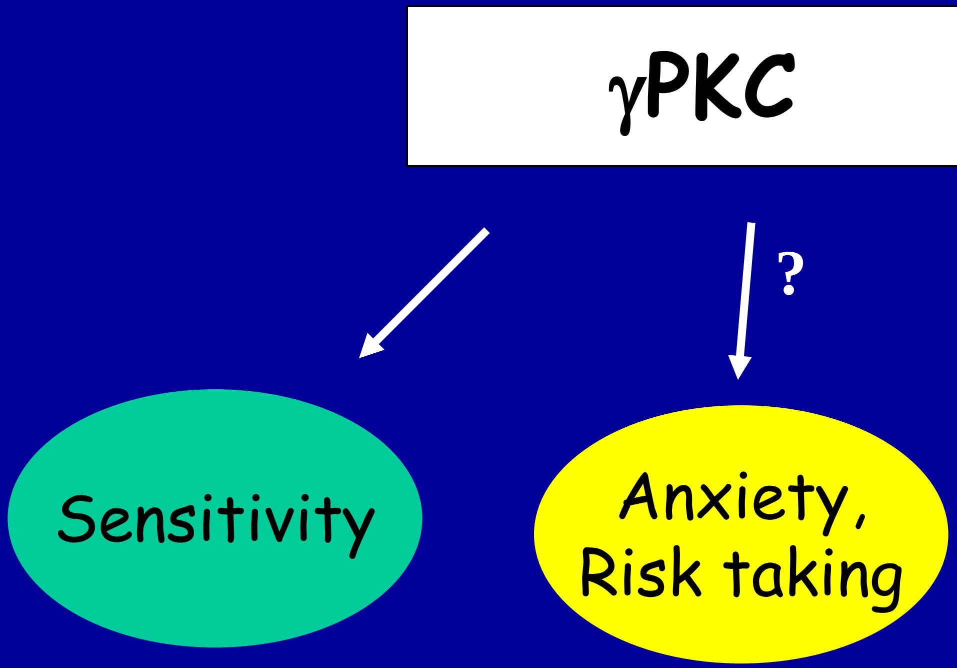
Correlation of ethanol modulation on GABA$_A$ responses
and duration of loss of righting reflex in PKC mouse lines
$PKC\gamma^{+/+}$
$PKC\gamma^{-/-}$
$PKC\varepsilon^{+/+}$
$PKC\varepsilon^{-/-}$
r^2=0.95
140
130
120
110
100
30
40
50
60
70
80
90
100
GABA$_A$ IPSC response (% of control)
Ethanol sleep time (min; 3.5 g/kg)

We conclude: γPKC and εPKC 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?

- γ–PKC mutants are also less sensitive to low-dose effects



Mutation leads to reduced sensitivity

Note: Novelty-seeking has been hard to model

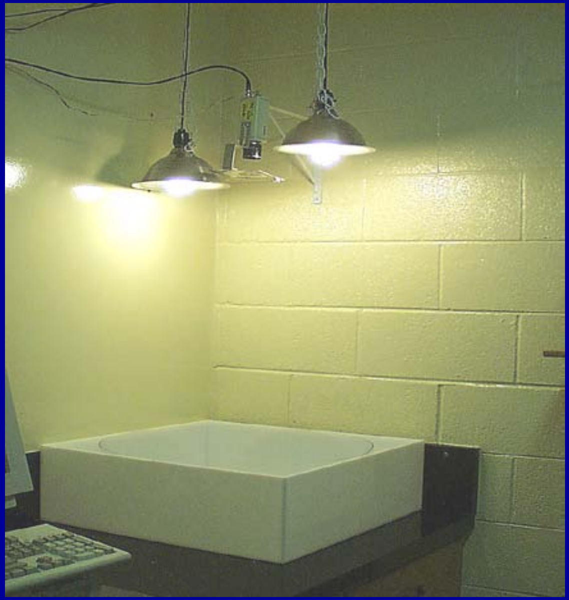
PKCγ null mutants may be risk takers...

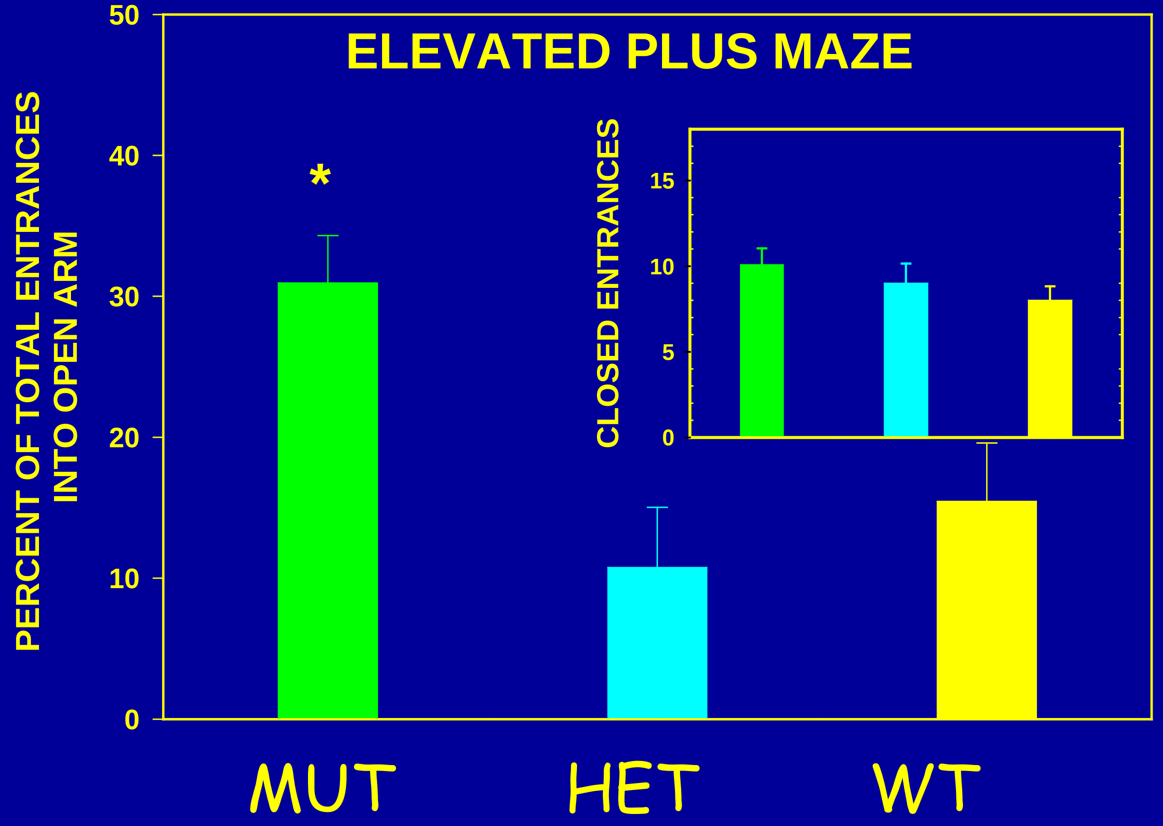
Slide from Jason Keller, Wehner lab

Elevated Plus Maze

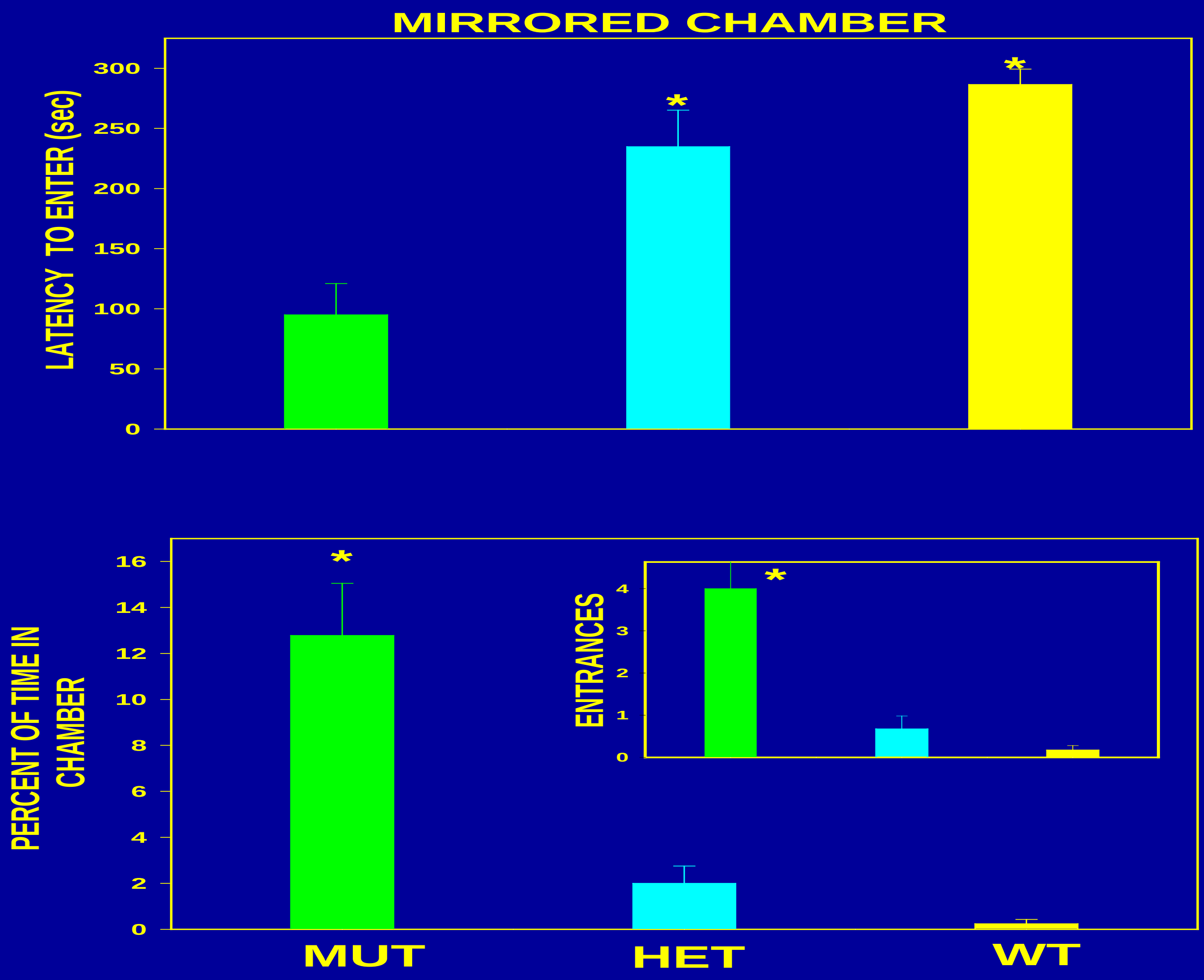
Mirrored Chamber Test

Open Field Arena

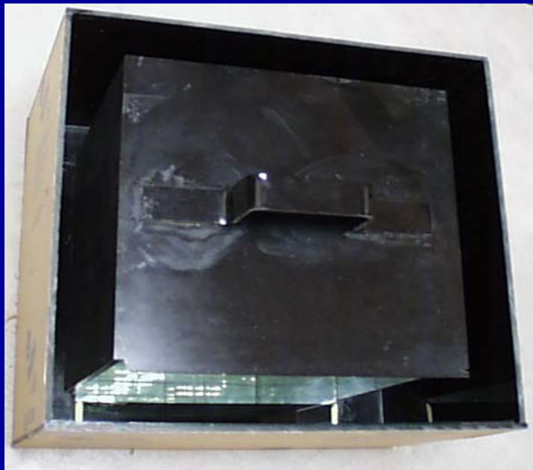




Mutants demonstrate less anxiety or greater exploration of novel places



γPKC mutants appear less anxious and again are willing to explore novel places

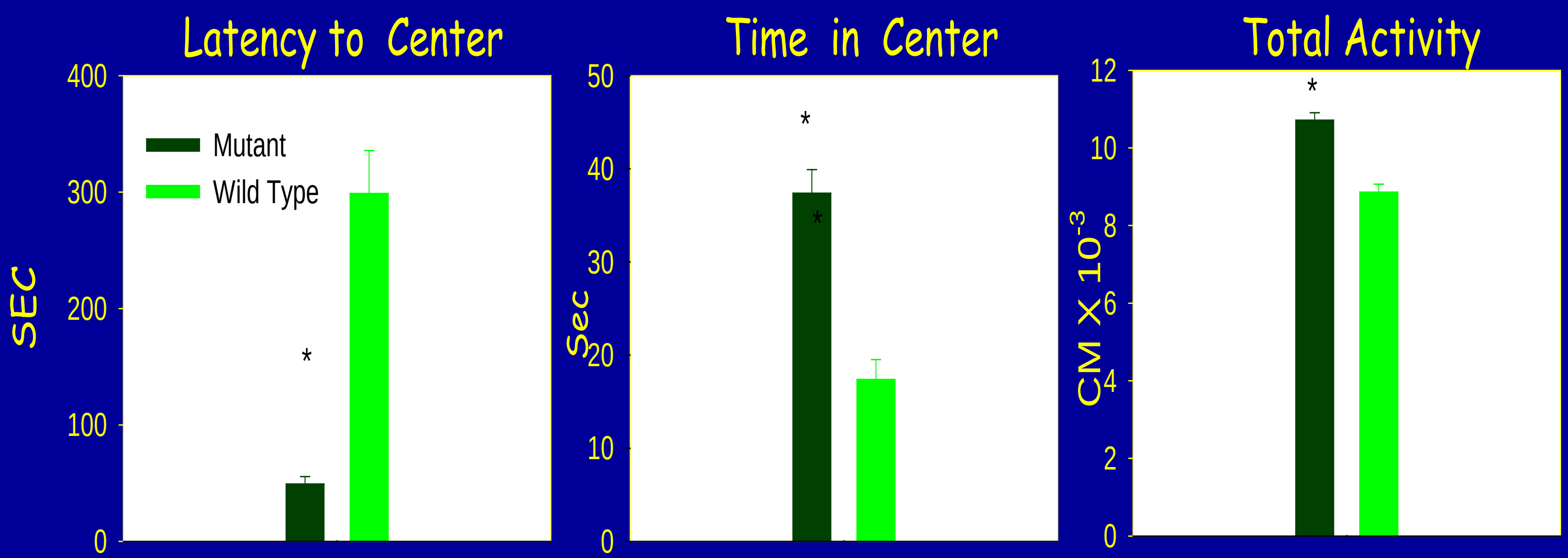


Open-field Studies

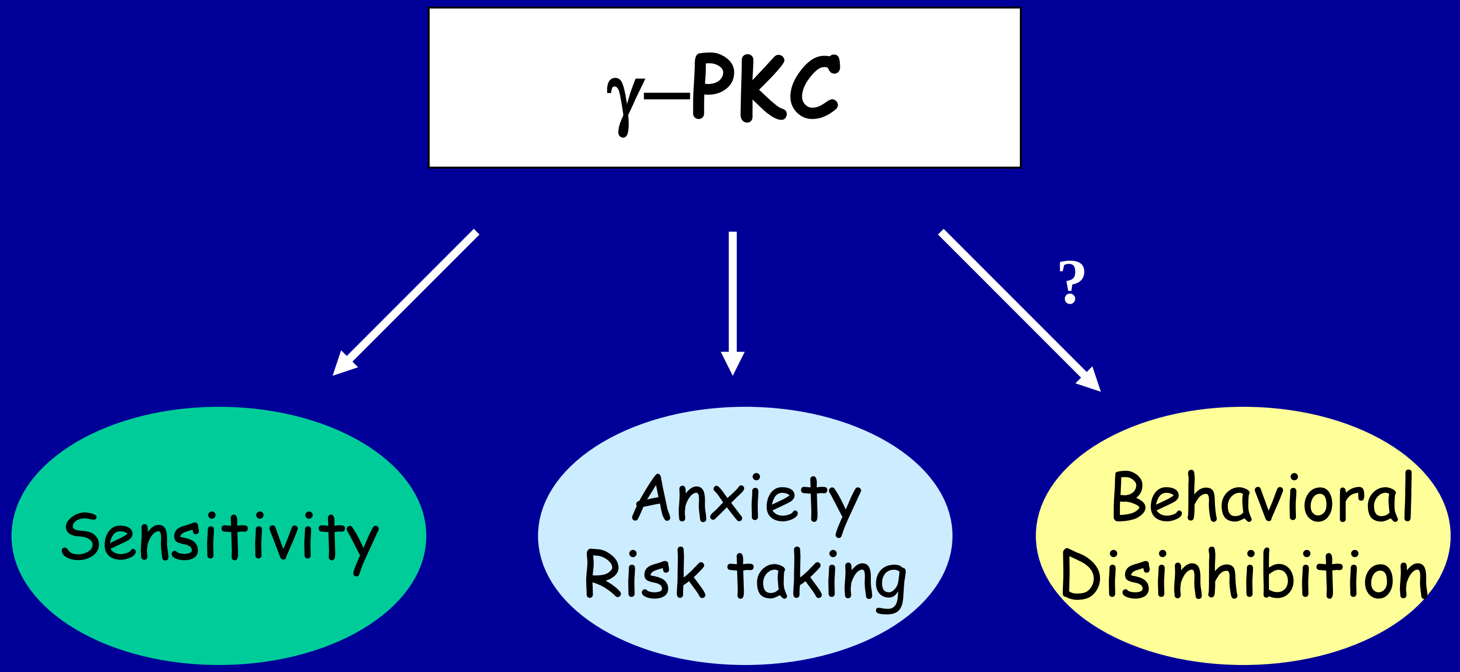
Security is a nice wall to hug!

That eagle will never get me. I am invincible!!!

Open-field behavior under white light in γ–PKC mice



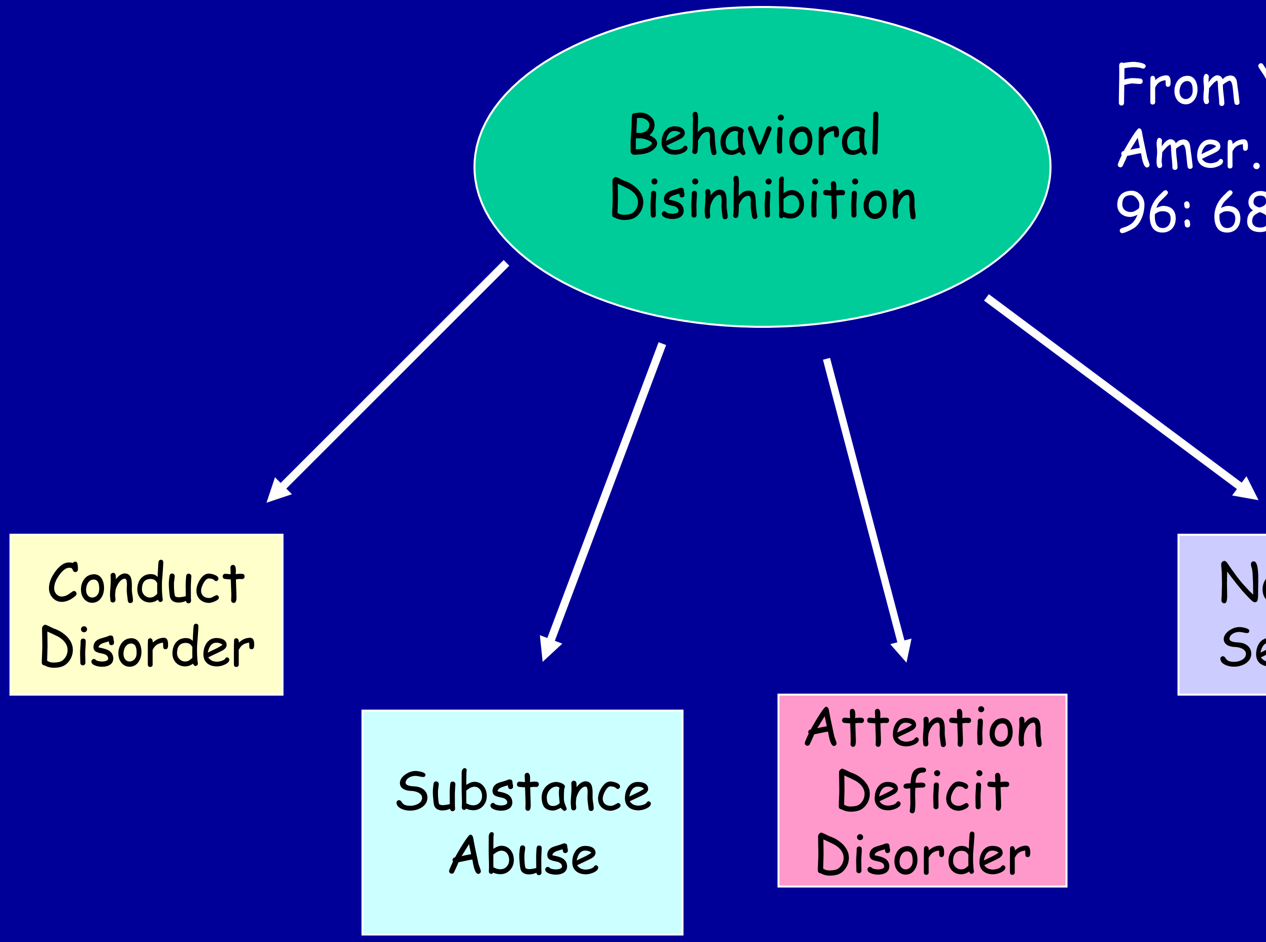
Mutants are more willing to explore center and spend more time there consistent with increased risk taking or less anxiety



Mutation leads to reduced sensitivity

Mutation leads to reduced anxiety or increased Risk taking

Human Genetic Modeling of Behavioral Disinhibition



From Young et al:
Amer. J. Med. Genetics
96: 684-695***

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

Measuring impulsivity in the mouse

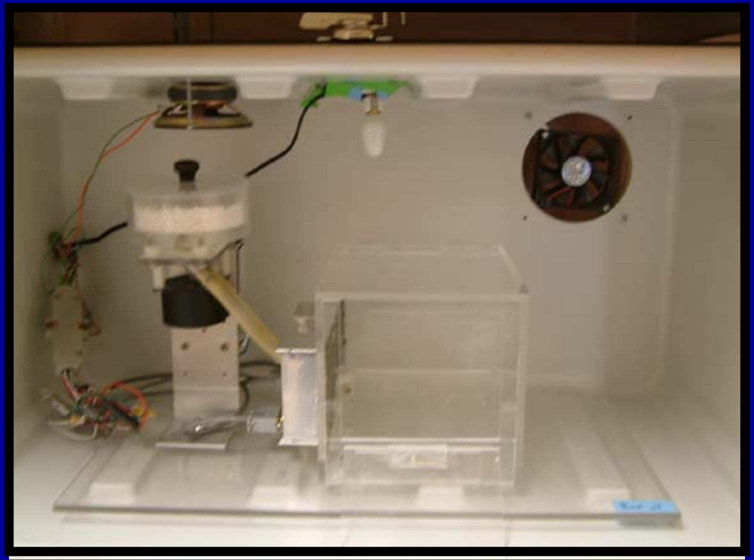
- Appetitive learning using an operant paradigm

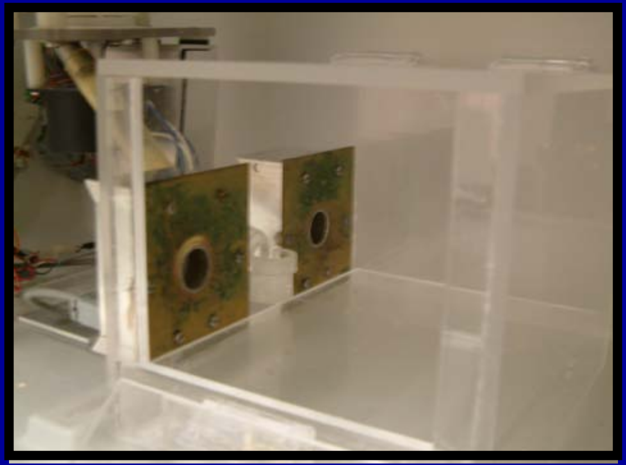
Slide from Dr. Barbara Bowers

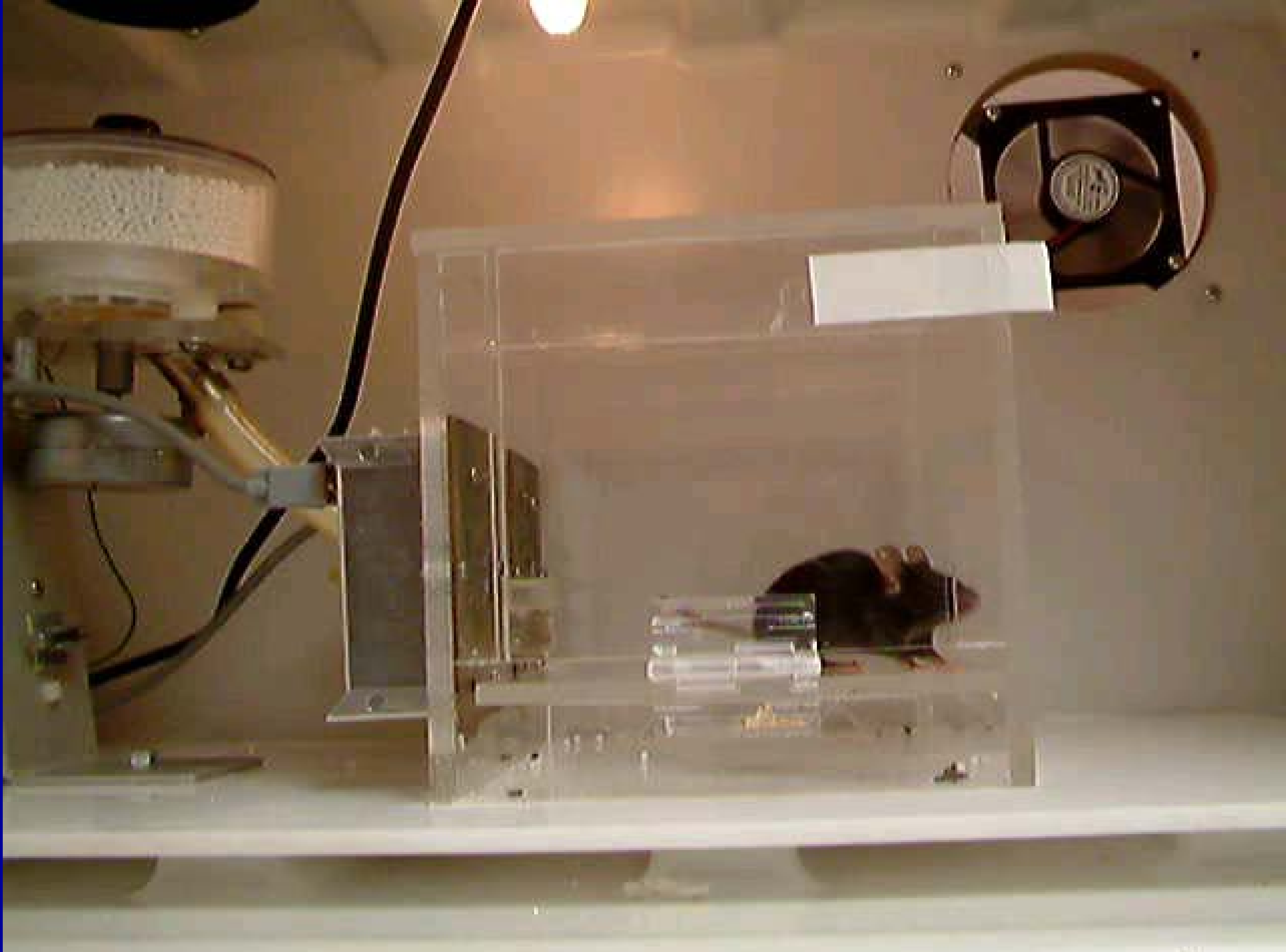
SIGNALED APPETITIVE TASK

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.

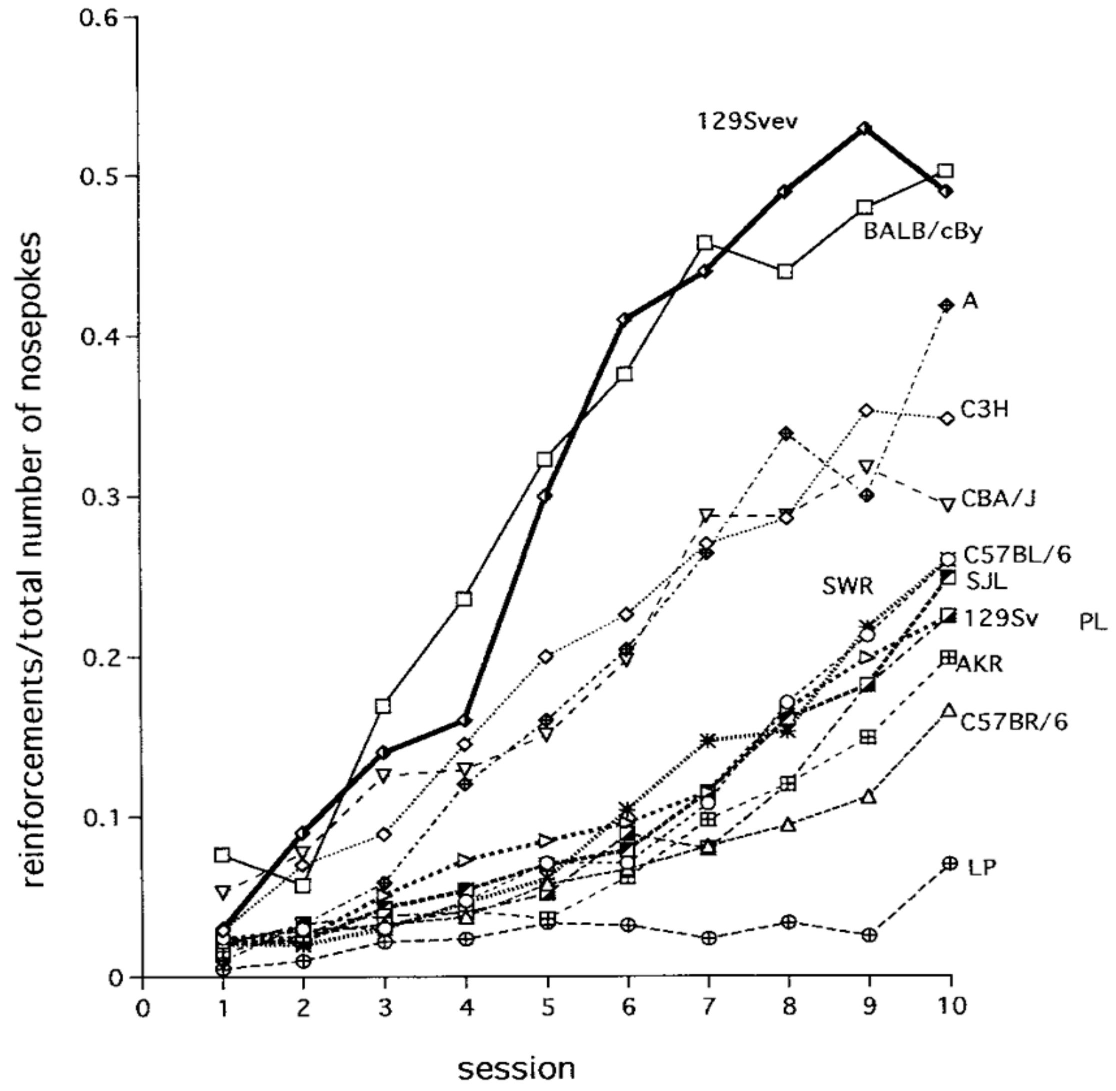




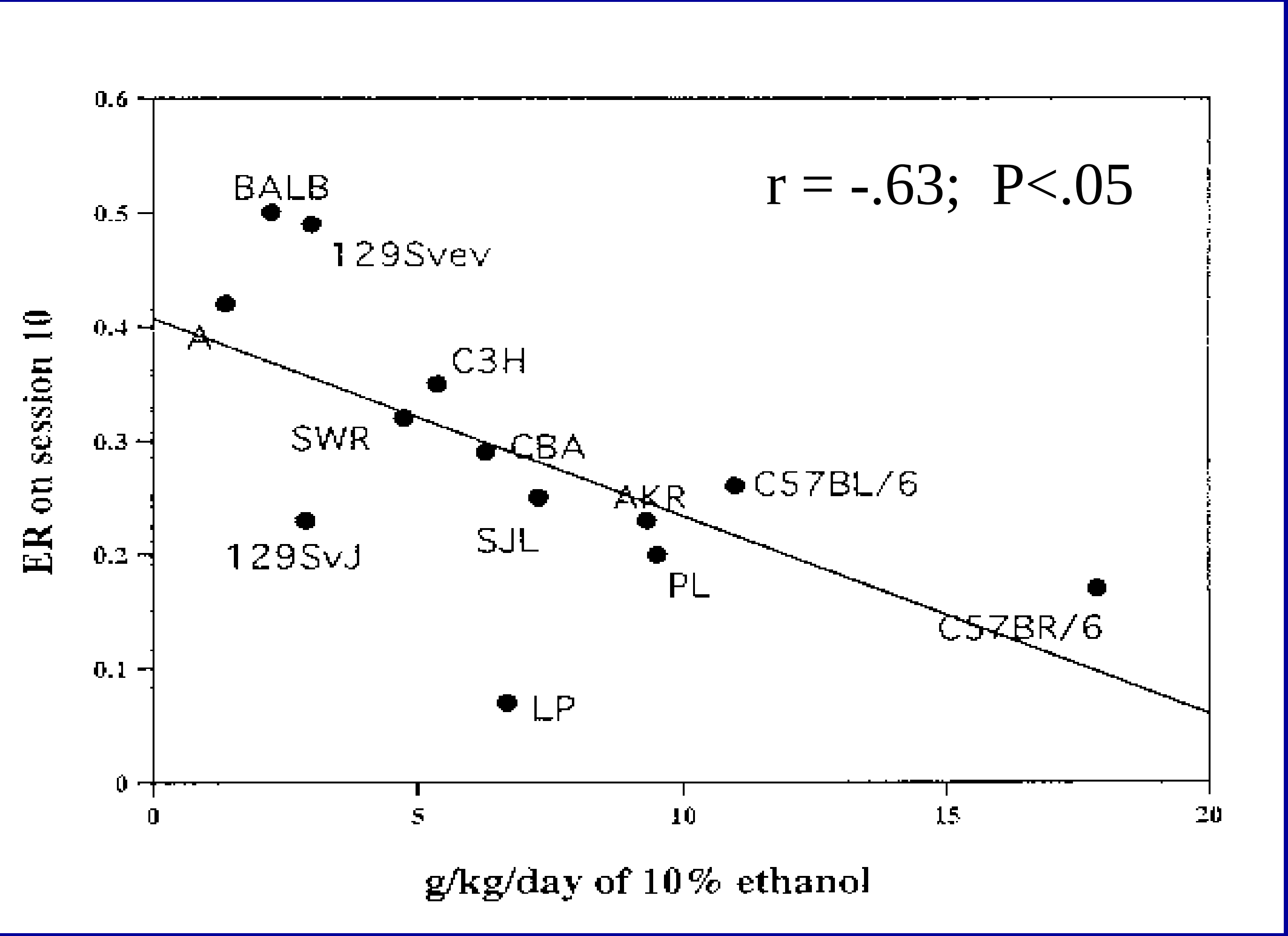


Efficiency Ratio for Withholding Responses for Impulsivity Task

Inbred Strain survey provides first evidence for genetic regulation of the withholding response



Impulsivity is negatively correlated with Ethanol consumption

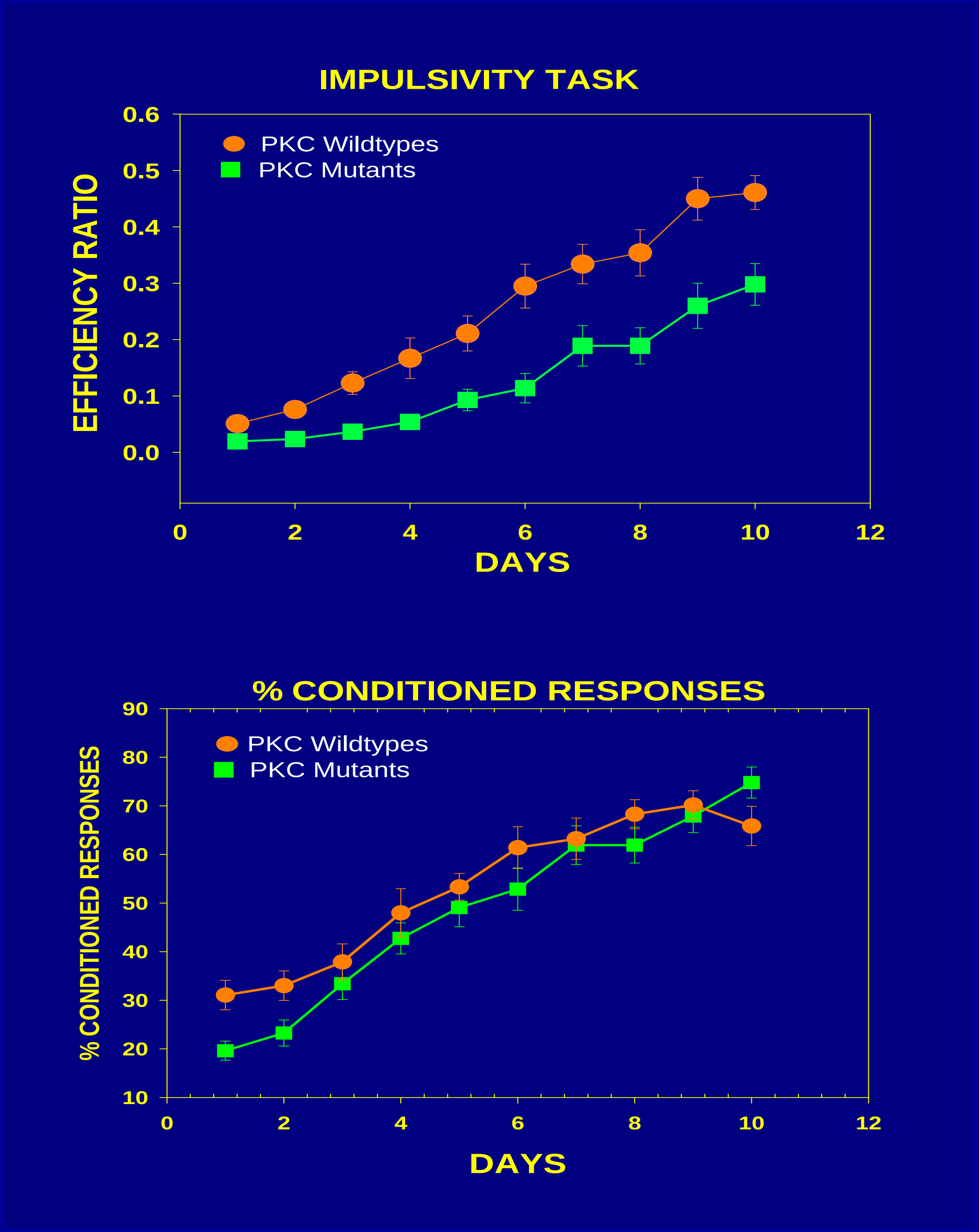


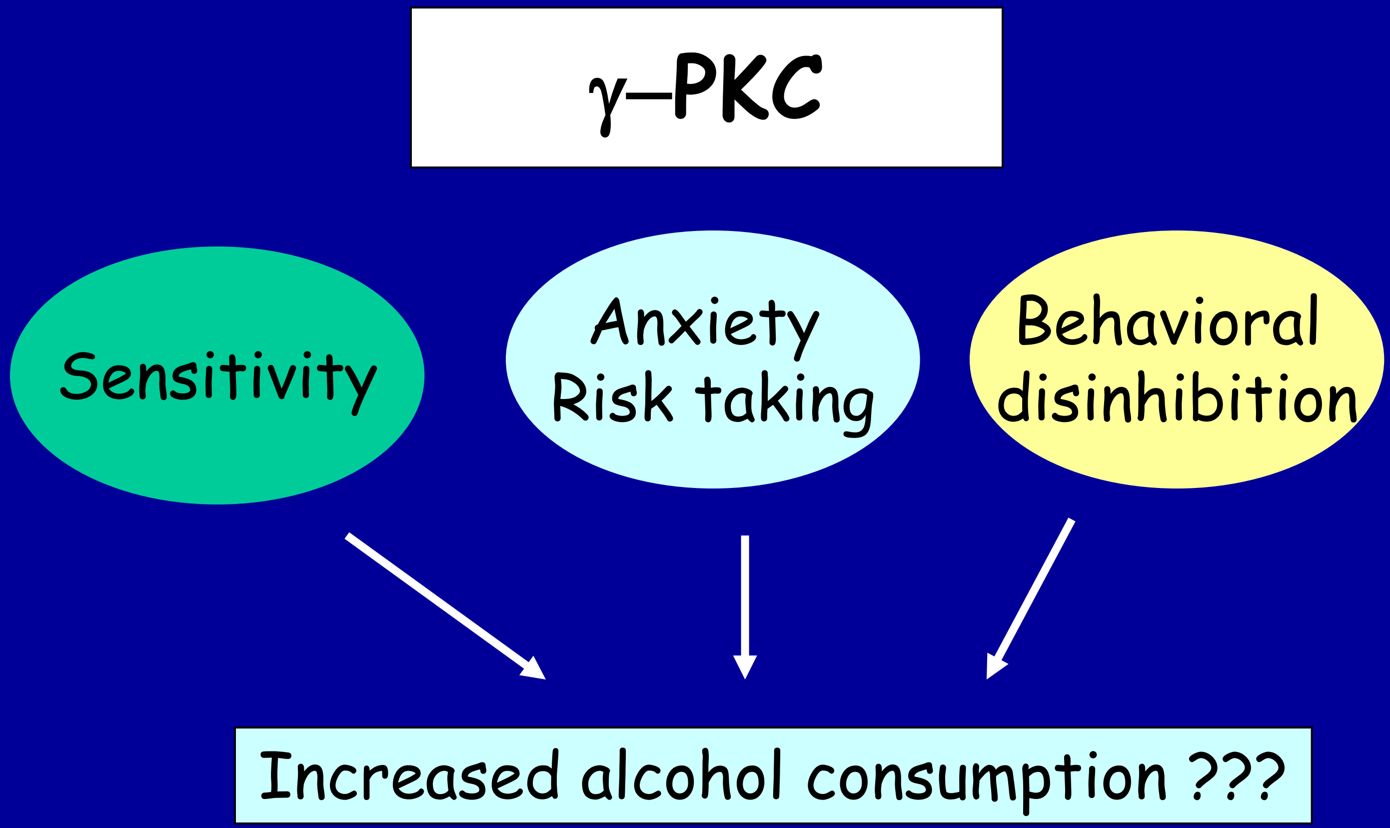
γ–PKC Null mutants are impaired on withholding responses to receive the sucrose reward

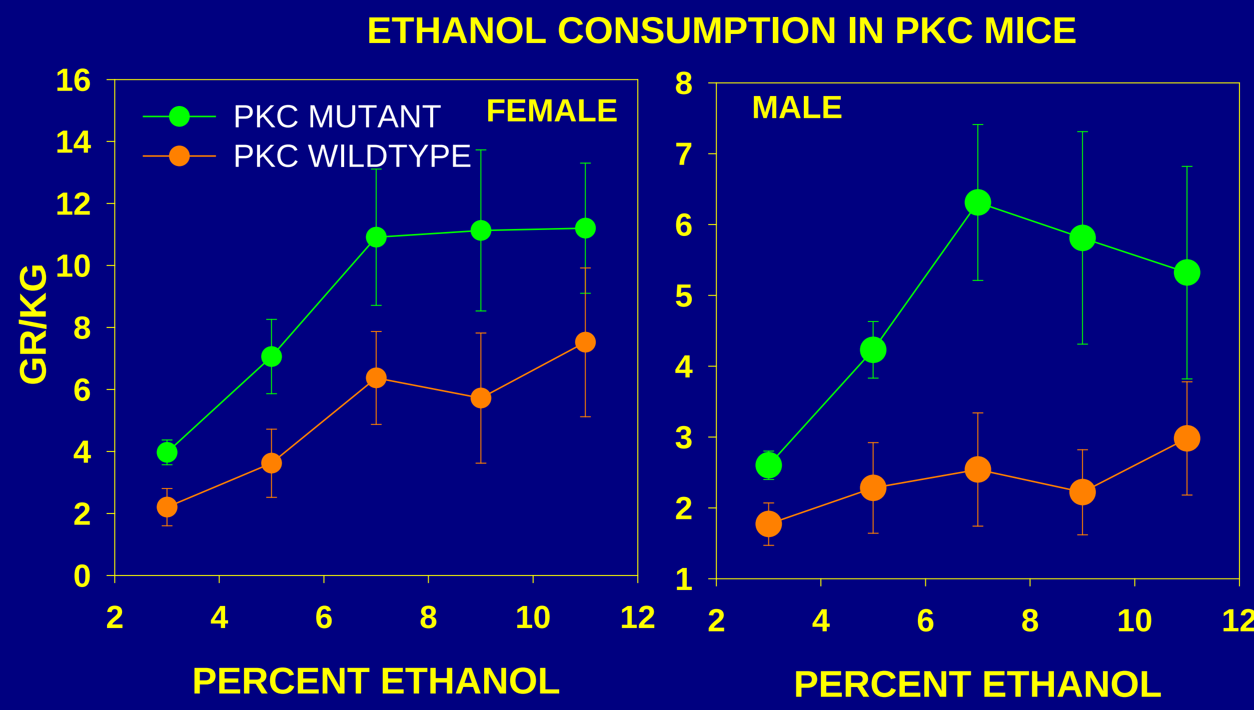
- What neurotransmitter system mediates this response?

  5HT 2 a/c receptors???- Bowers

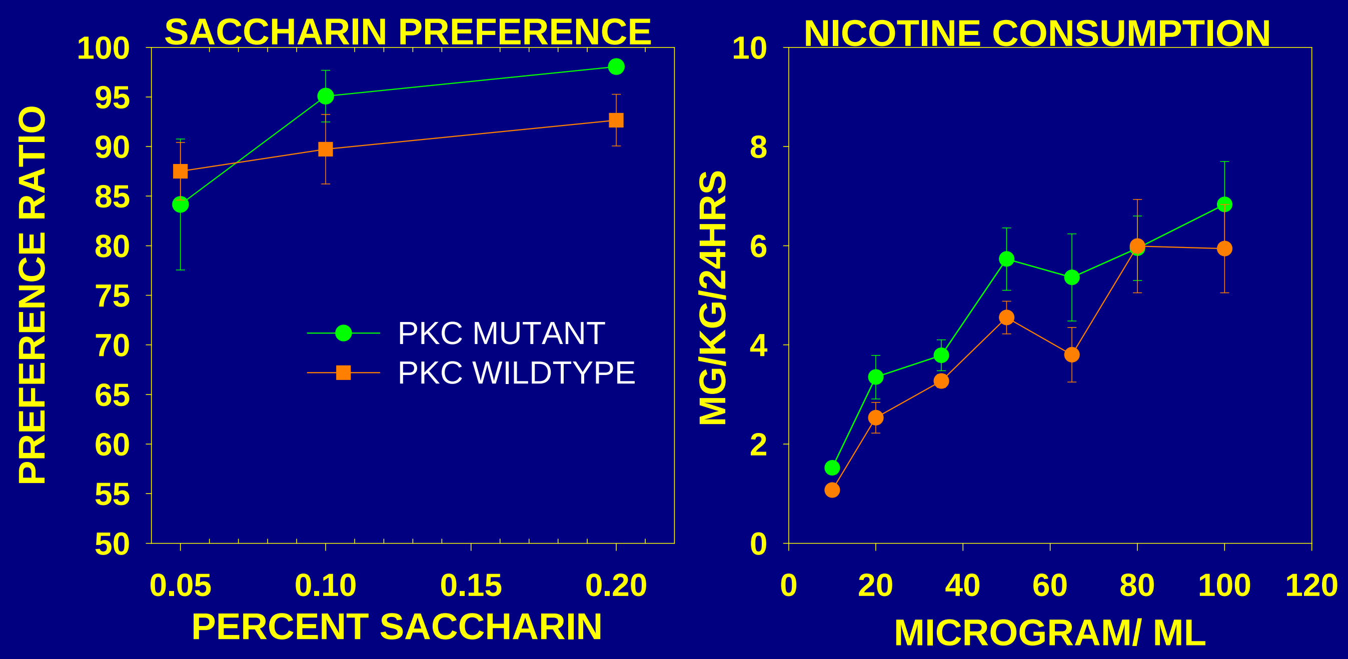
Bowers and Wehner (2001) J.Neuroscience: 21: RC180 (1-5)



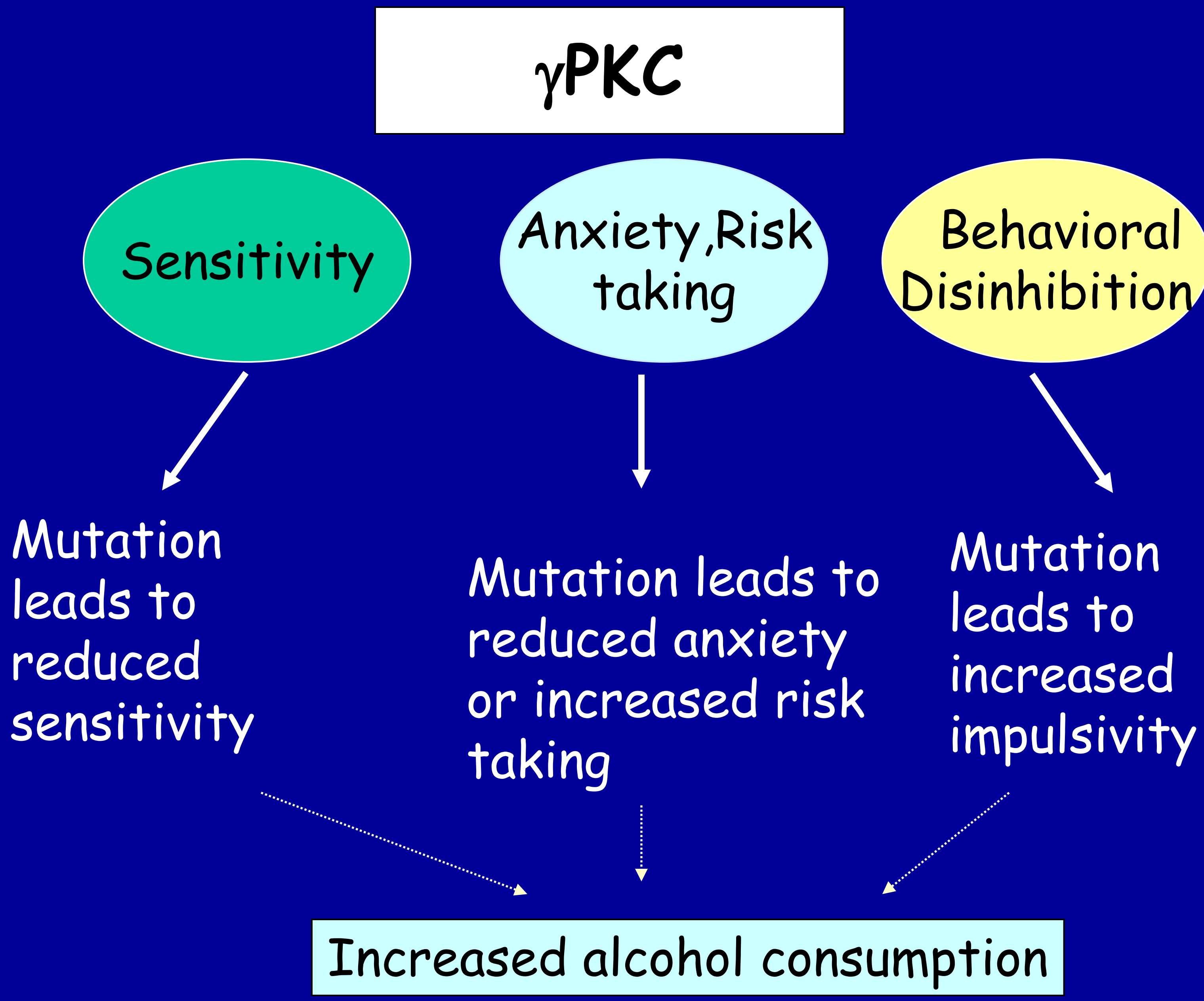

γ–PKC
Sensitivity
Anxiety
Risk taking
Behavioral
disinhibition
Increased alcohol consumption ???



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


γPKC
Sensitivity
Anxiety,Risk taking
Behavioral Disinhibition
Mutation leads to reduced sensitivity
Mutation leads to reduced anxiety or increased risk taking
Mutation leads to increased impulsivity
Increased alcohol consumption

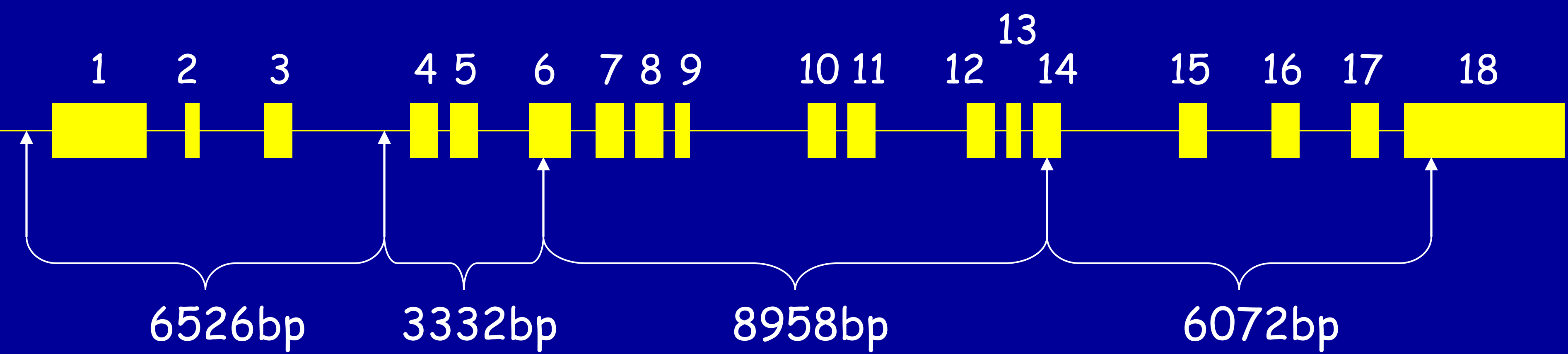
Conclusions about γ–PKC

γ-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 γ-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:

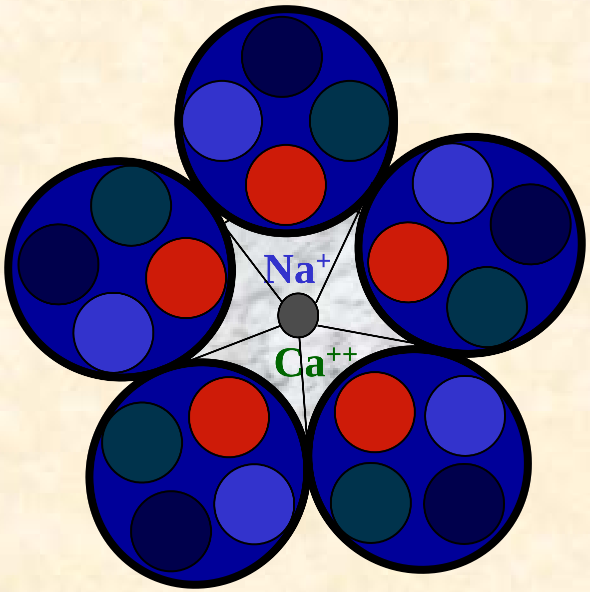
- Collaborative work with Allan Collins, IBG
- The role of nicotinic receptors in mediating sensitivity to ethanol's effects the startle response

Background for study of nicotine and ethanol on 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 *in vitro*

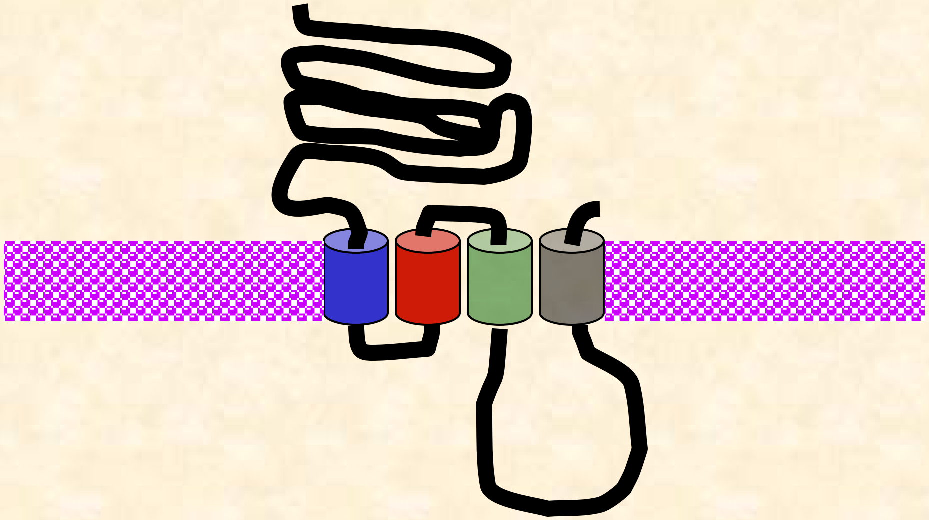
NEURONAL NICOTINIC RECEPTOR STRUCTURE

A.



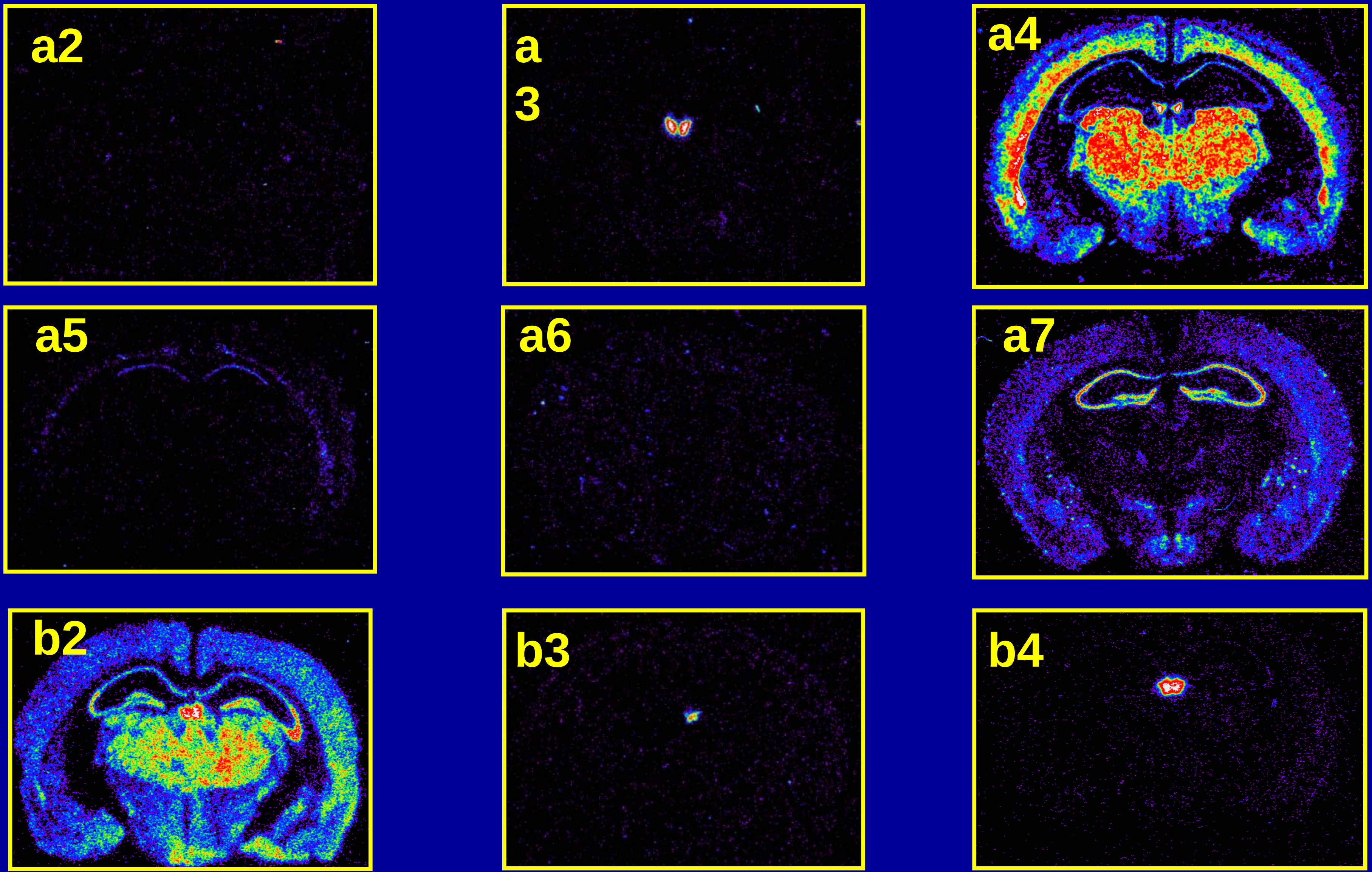
Alpha 2, 3, 4, 5, 6, 7..9,10
Beta 2, 3, 4

B.



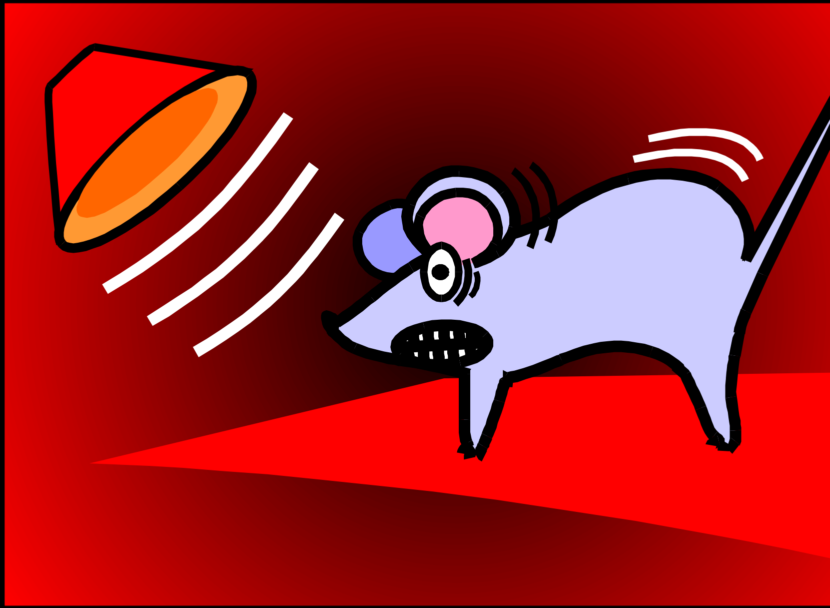
A4β2* highly expressed in Brain

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

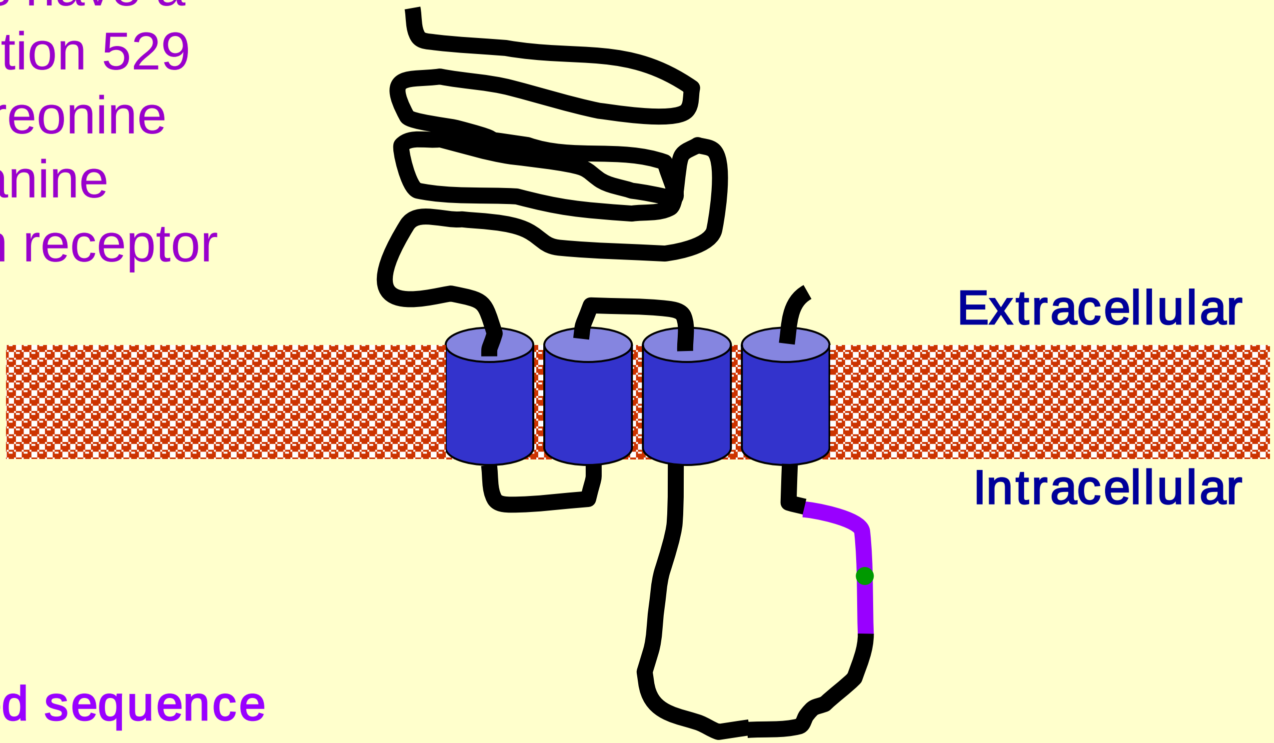
Drawing from Dr. Karen Stevens

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

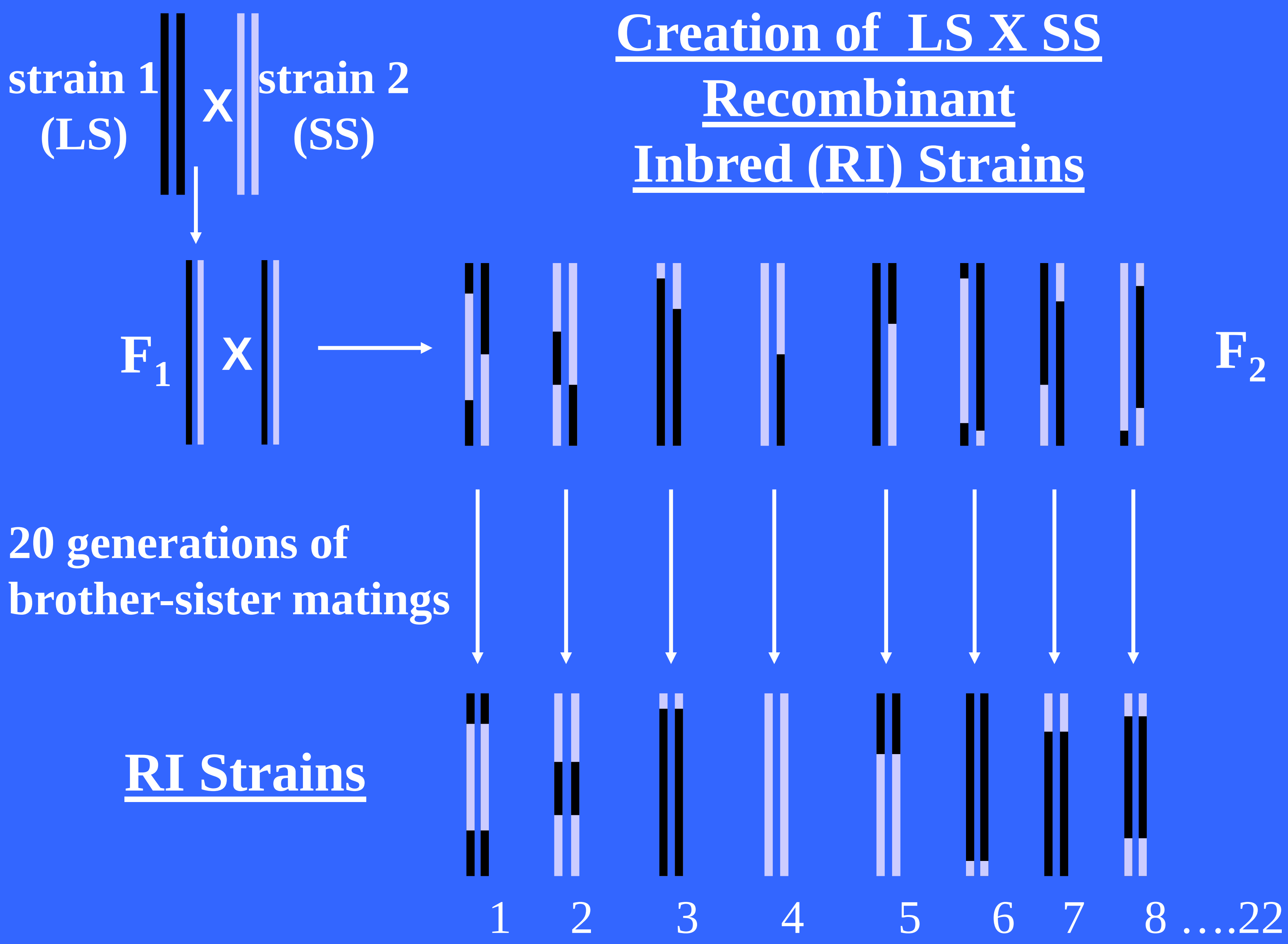


▬ Region of displayed sequence

GAASLTESKPTGSPASLKTRPSQLPVSDQ**T**SPCKCTCKEPSPVSPITVLKAGGTKAPPQHLP

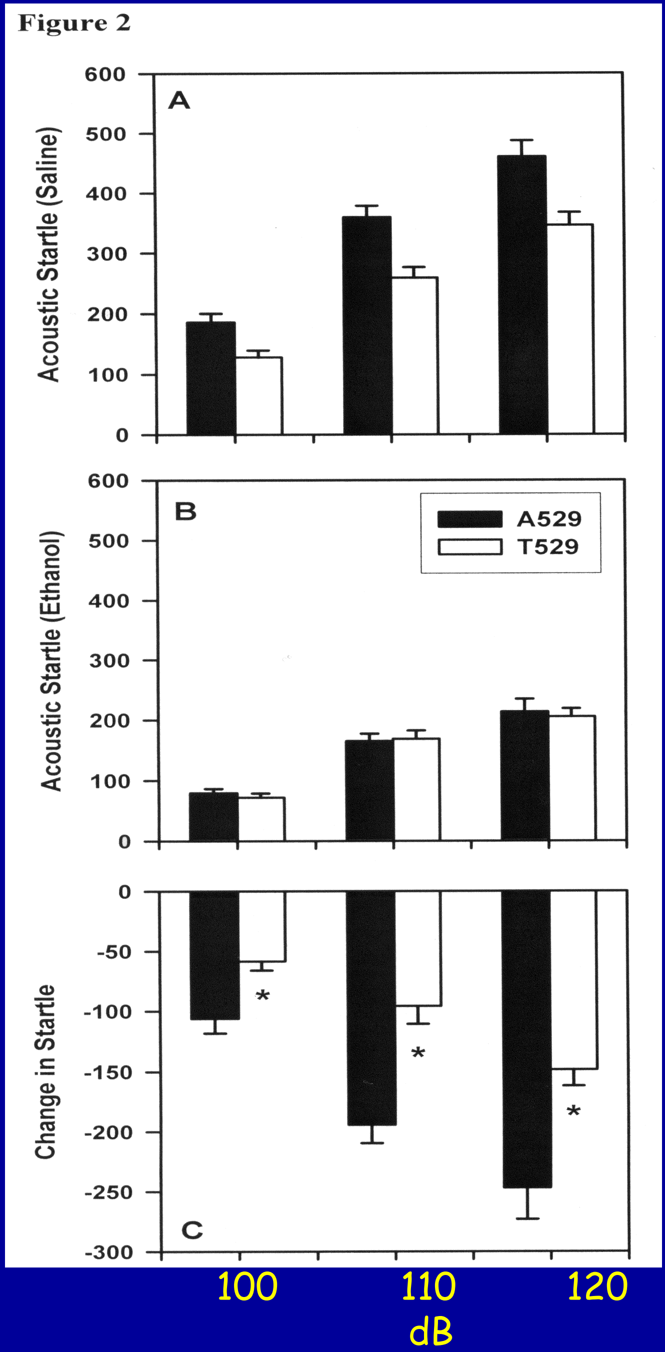
GAASLTESKPTGSPASLKTRPSQLPVSDQ**A**SPCKCTCKEPSPVSPITVLKAGGTKAPPQHLP

From: Dr. Jerry Stitzel, Institute for Behavioral Genetics


Creation of LS X SS Recombinant Inbred (RI) Strains
strain 1 (LS)
X
strain 2 (SS)
F1
X
F2
20 generations of brother-sister matings
RI Strains
1 2 3 4 5 6 7 8 ….22

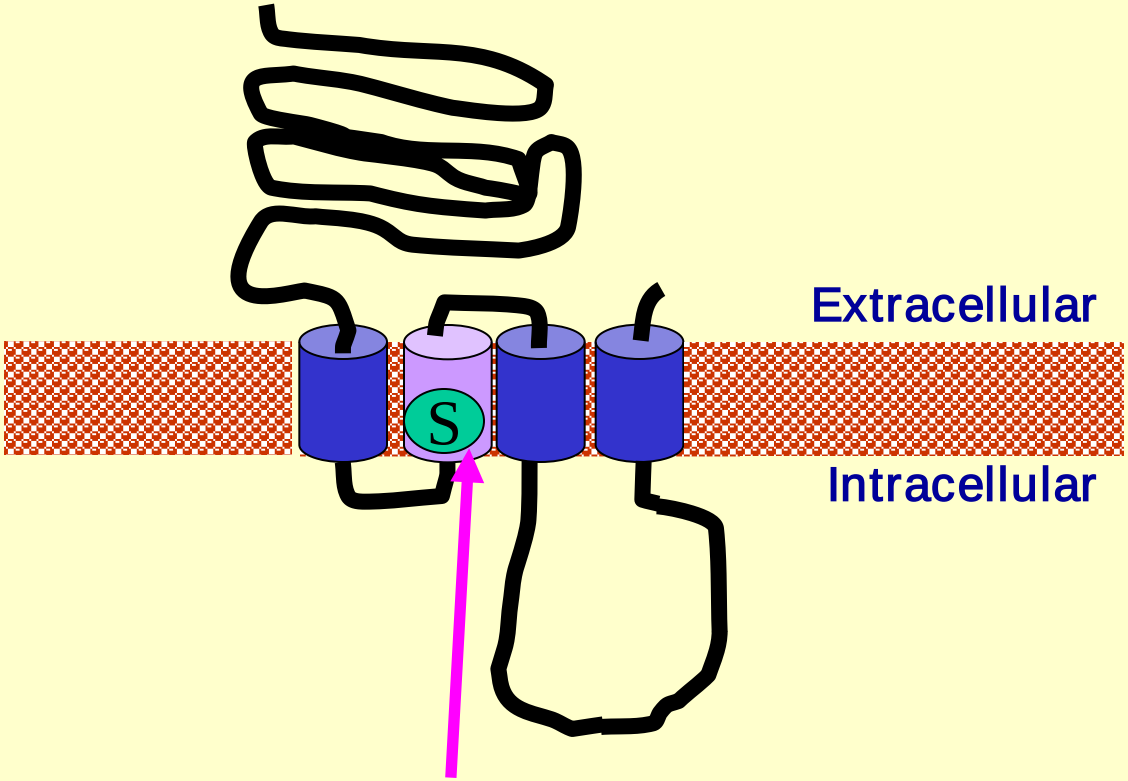
Results in LSXSS Recombinant Inbreds

- 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 α4-containing receptors in mediating the effects of ethanol on startle
- Animal models were needed to test this role of α4-containing receptors more directly.



Gain of Function Mutation in α4 Nicotinic Subunit

- In brain usually in heteromer as α4β2
  - Acetycholine
  - 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.

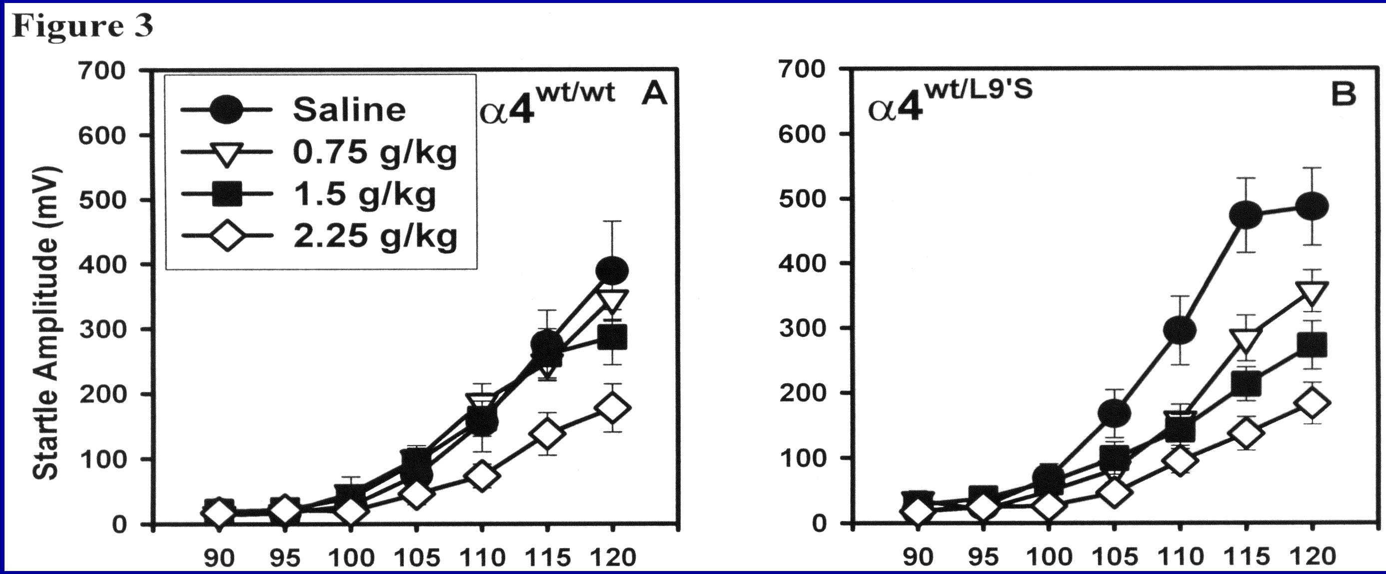
Prediction:

Gain of function mutants should be MORE sensitive to ethanol

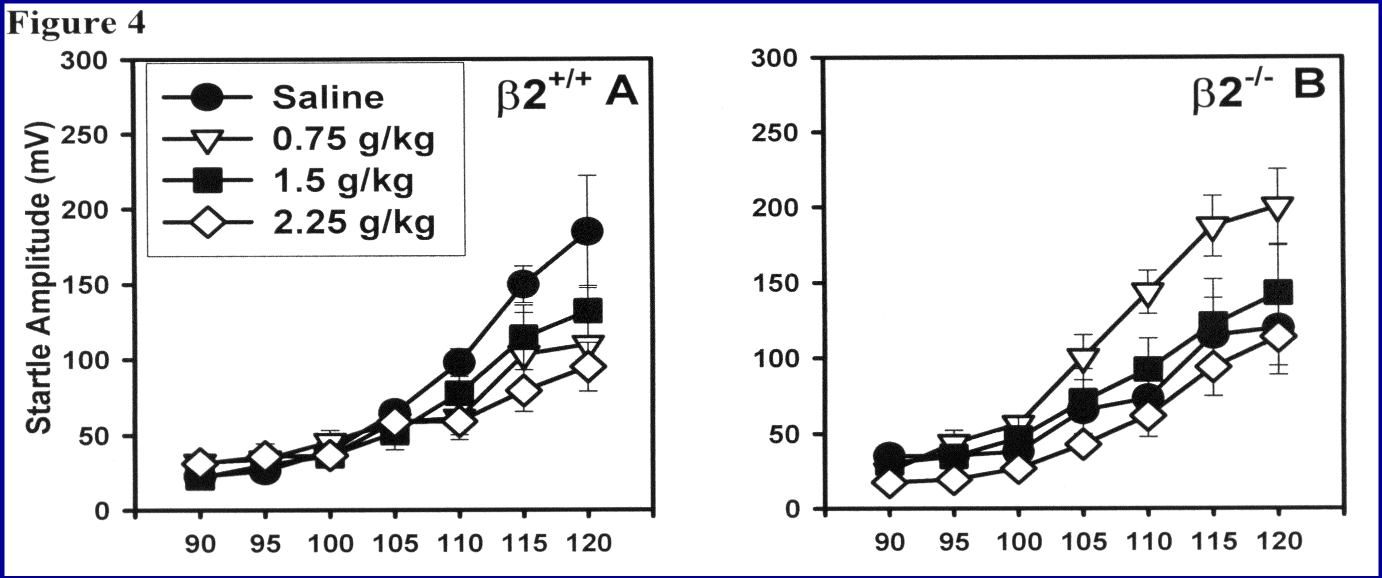
Null mutants should be LESS sensitive to ethanol

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

- $\alpha4\beta2$-containing receptors may play important roles in modulating the effects of ethanol and nicotine on acoustic startle response

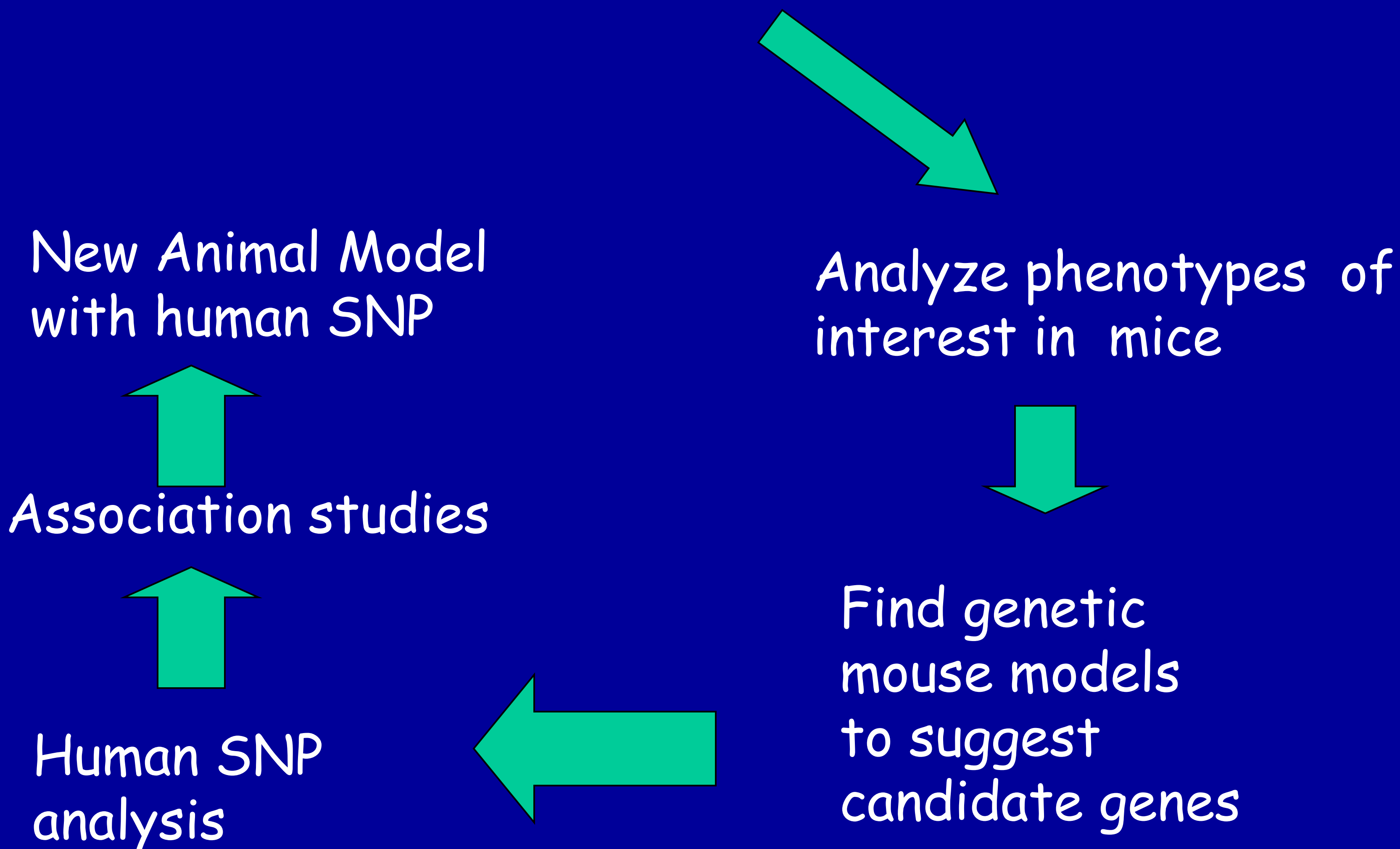
- Evaluate the A529T $\alpha4$ subunit polymorphism using a knock-in mouse line

Drs. Gregg Homanics (PITT) and Jerry Stitzel (IBG)

<u>Translating this to humans</u>

Dr. Marissa Ehringer: examining nicotinic gene family
Dr. Kent Hutchinson: $\alpha4$ with startle response

Alcoholism


Analyze phenotypes of interest in mice
Find genetic mouse models to suggest candidate genes
Human SNP analysis
Association studies
New Animal Model with human SNP

Contributors to the work

PKC WORK

Dr. Barbara Bowers
Dr. Sheree Logue
Denise Hix
Jill Miyamoto
Jason Keller

Nicotinic Work

Dr. Allan Collins
Dr. Jeremy Owens
Dr. Seth Balogh

Other CU labs

Dr. William Proctor (UCHSC)
Dr. Marissa Ehringer
Dr. Jerry Stitzel

Mutant lines

Dr. Asa Abeliovich
Dr. Susumu Tonegawa
Dr. Robert Messing
Dr. Henry Lester
Dr. Marina Picciotto

FUNDED by NIAAA