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Animal models of ethanol and nicotine interactions

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- Animal Models of Ethanol and Nicotine Interactions

The Human Model

- 70-80% of alcoholics are smokers.
- Alcoholics smoke more cigarettes per day than do non-drinking smokers.
- Approximately 40% of smokers are alcoholics or alcohol abusers.
- Lab experiments: smoking increases alcohol consumption and vice versa.

Is it possible to develop a comprehensive animal (mouse) model of alcoholism or smoking?

NO

The behavioral geneticist's mantra:

$$V_p = V_G + V_E + V_{GxE}$$

- Human studies suggest genetic influence on alcohol abuse and smoking.
- There may be common genes that affect both forms of substance abuse.
- Shouldn't an animal model consider genetic issues? Willy-nilly selection of "a rat" or "a mouse" might mean a non-drinker or non-smoker is being modeled.

FORWARD GENETICS

- Relies on genetically-mediated variation in a population
- The goal is to identify polymorphisms that contribute to this variation
- The “answer” obtained depends on the population studied (if the animal studied does not have a “poly” in an important gene forward genetics will fail to detect a role for that gene)
- Can be slow, time consuming, frustrating

REVERSE GENETICS

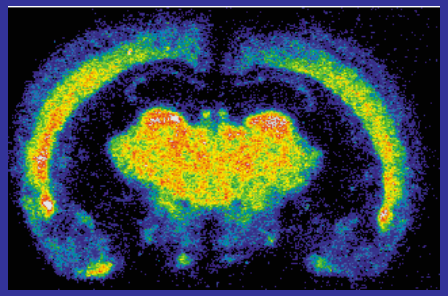
- Goal is to test the role of candidate genes in regulating a phenotype. The method is a gamble with potential for big payoff.
- Results are not always straightforward and changes in phenotype could be due to compensatory changes, developmental effects, etc.

The pharmacologist's mantra

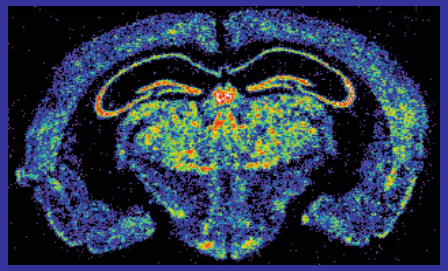
D + R → DR → Response

- Questions that we have addressed:
  - Do nicotinic receptors modulate normal behaviors?
  - Do nicotinic receptors modulate nicotine-related behaviors?
  - Do nicotinic receptors modulate alcohol-related behaviors?

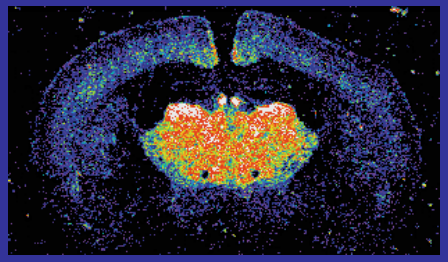
α4β2 nAChRs are found throughout the CNS



α4 *in situ* hybridization



β2 *in situ* hybridization



[^{3}H]Nicotine binding

A Pharmacologist's (A. Goldstein) View of Components of Addiction

- Reinforcement (+ and -)
- Initial sensitivity
- Tolerance/sensitization
- Withdrawal

The Sensitivity Model

- High sensitivity to positive actions increases vulnerability to addiction.
- Low sensitivity to toxic actions increases vulnerability to addiction.
  - Low sensitivity could be innate (genetically determined)
  - Low sensitivity could be acquired (drug tolerance and/or environmental mediation).
  - Could be due to altered metabolism or CNS sensitivity.

STUDIES WITH INBRED STRAINS LED TO THE POSTULATE THAT ANIMALS WITH MORE NICOTINIC RECEPTORS HAVE GREATER SENSITIVITY TO NICOTINE.

D + R → DR → Response

Do Common Genes Influence Nicotine and Alcohol Actions?

- We started with the LS-SS mice that were selectively bred for DIFFERENCES in sensitivity to high doses of alcohol.
- LS-SS also differ in sensitivity to low dose effects of alcohol.
- LS-SS differ in alcohol withdrawal.
- LS-SS do not differ in oral alcohol intake.

LS and SS Mice and NICOTINE

- LS-SS Mice Differ in Sensitivity to Nicotine
  - Open field activity
  - Y-maze activity
  - Body temperature
  - Anxiety
  - Seizures
  - Acoustic startle

    No difference in number of nicotinic receptor binding sites
  - No difference in nicotine metabolism

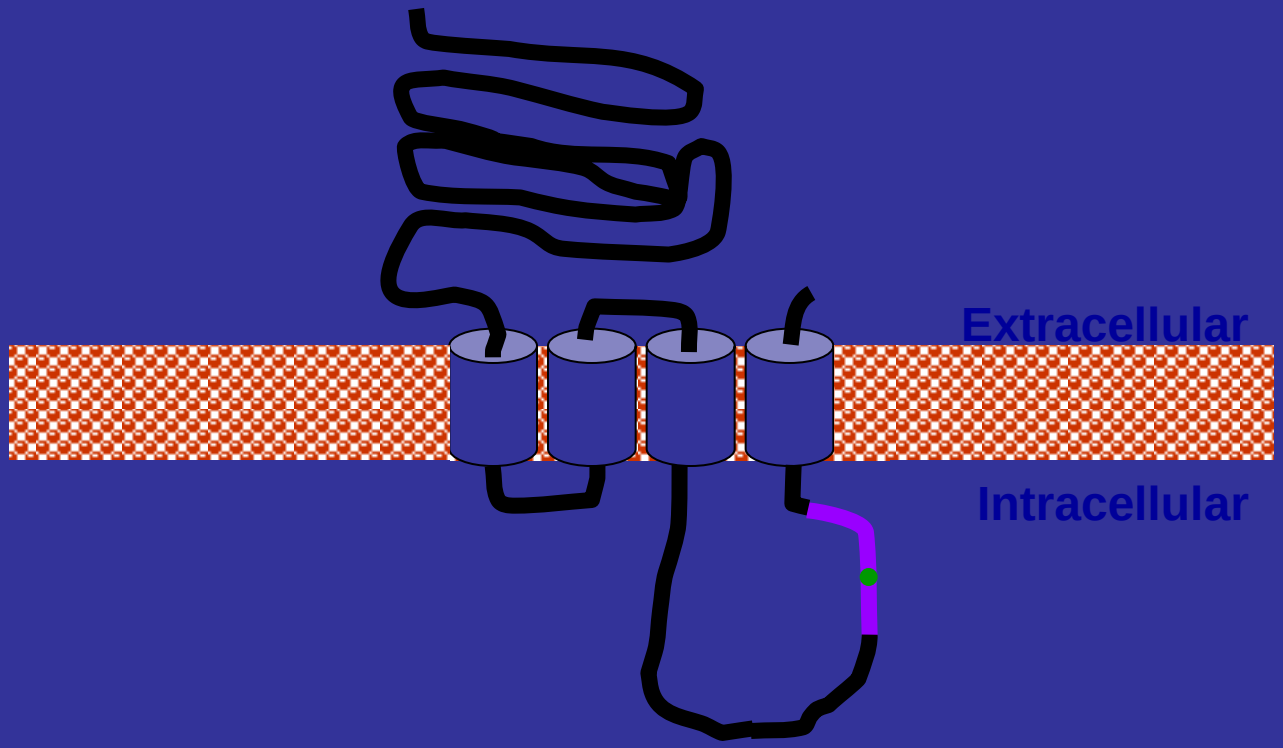
Interpretations of LS-SS Results

- Differences in sensitivity to nicotine could mean that nicotine genes are also alcohol genes.
- Nicotine differences could reflect unwanted effects of inbreeding (small colony), linkage to “alcohol” genes, etc.

Do LS-SS nicotinic receptors Differ?

- No difference in [^{3}H]-nicotine binding.
- No difference in [^{125} I]-α-BTX binding EXCEPT in cerebellum.
- Are there any differences in receptor structure (e.g. amino acid sequence) that produce differences in receptor function?

Location of α4 Missense Mutation in Mice

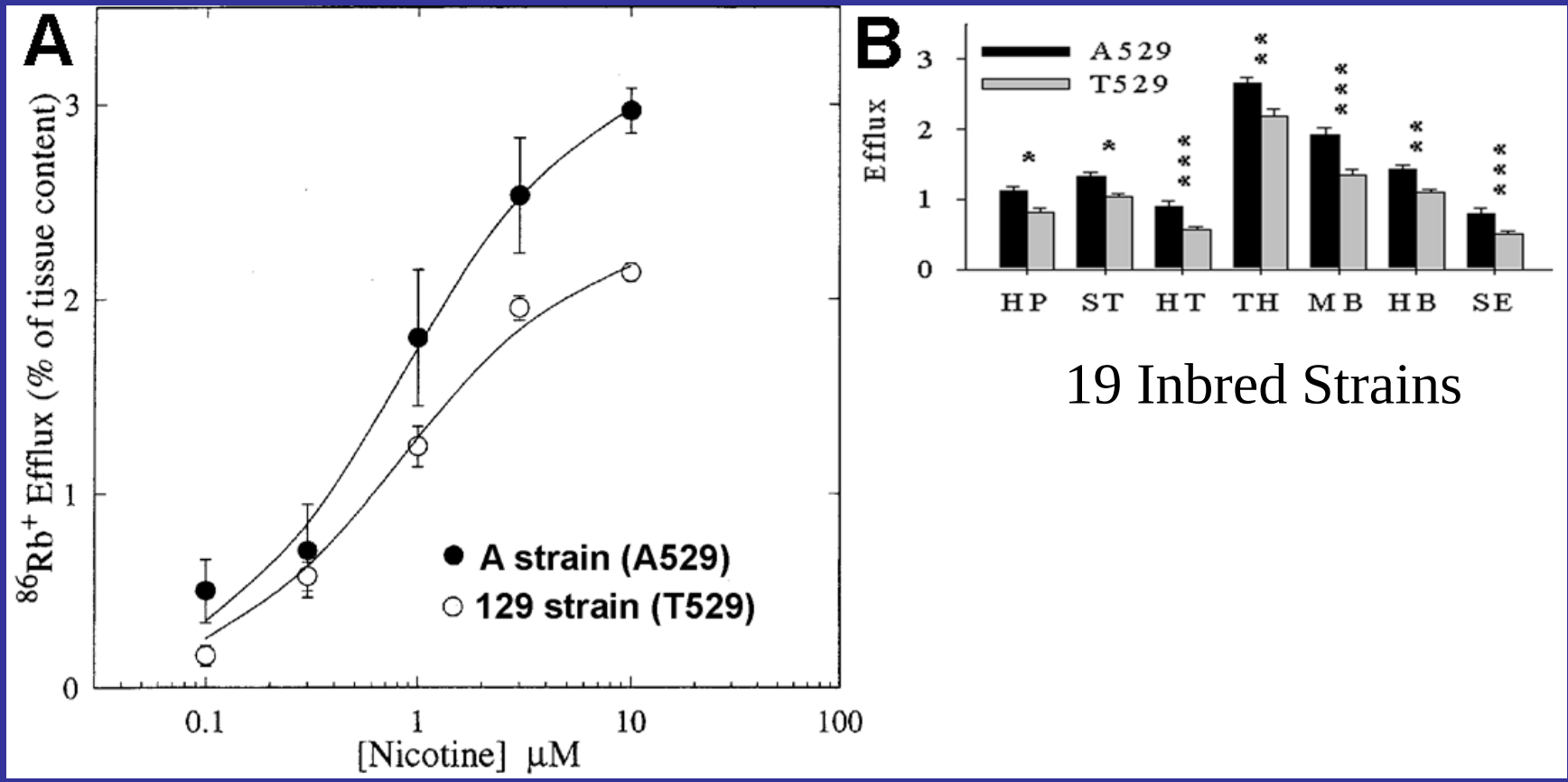


GAASLTESKPTGSPASLKTRPSQLPVSDQTSPCKCTCKEPSPVSPITVLKAGGTKAPPQHLP
GAASLTESKPTGSPASLKTRPSQLPVSDQASPCKCTCKEPSPVSPITVLKAGGTKAPPQHLP

Does the α4 Polymorphism Change Receptor Function?

- Receptor function can be measured using an ion ($^{86}Rb^{+}$) efflux assay
- Can also measure function by monitoring neurotransmitter release (dopamine, GABA)

A/T Differences in $^{86}Rb^{+}$ flux



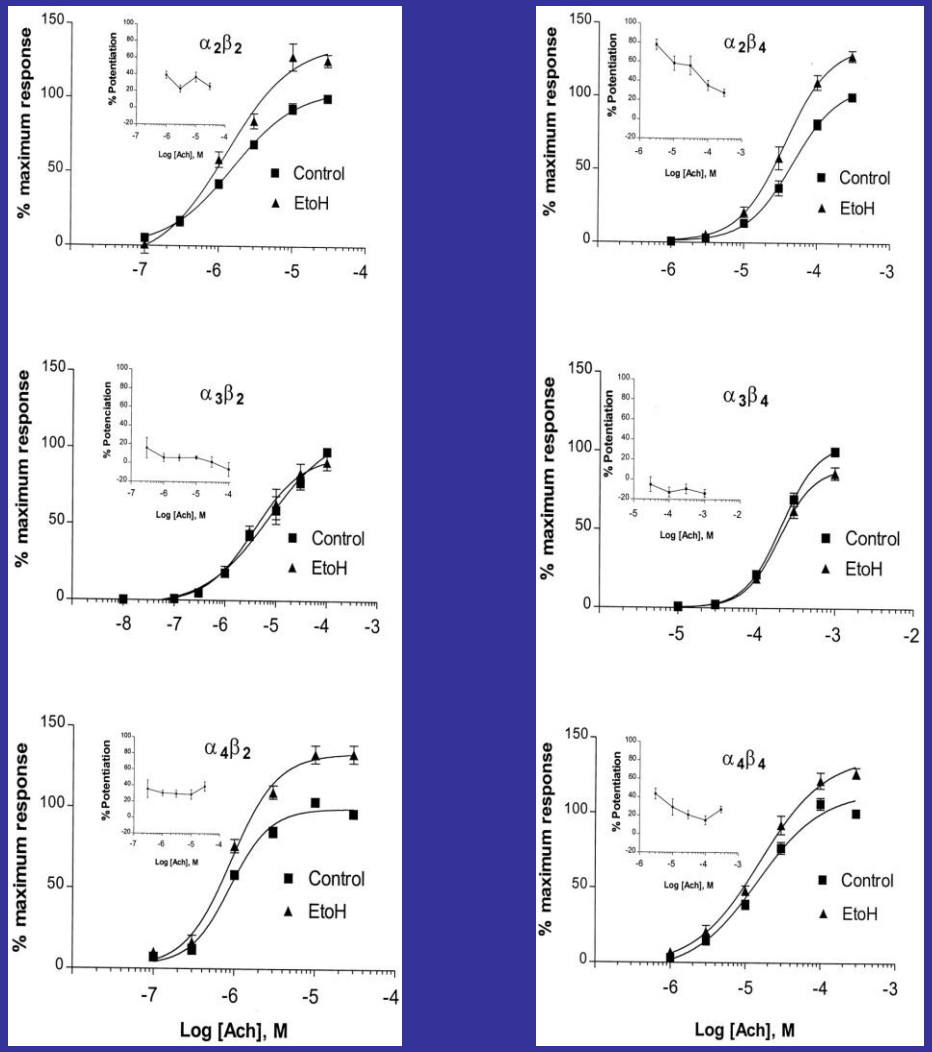
19 Inbred Strains

Dobelis *et al*. (2002) *Mol. Pharmacol.* 62: 334-42.

EtOH Enhances the Function of Some Combos of Ectopically Expressed nAChRs

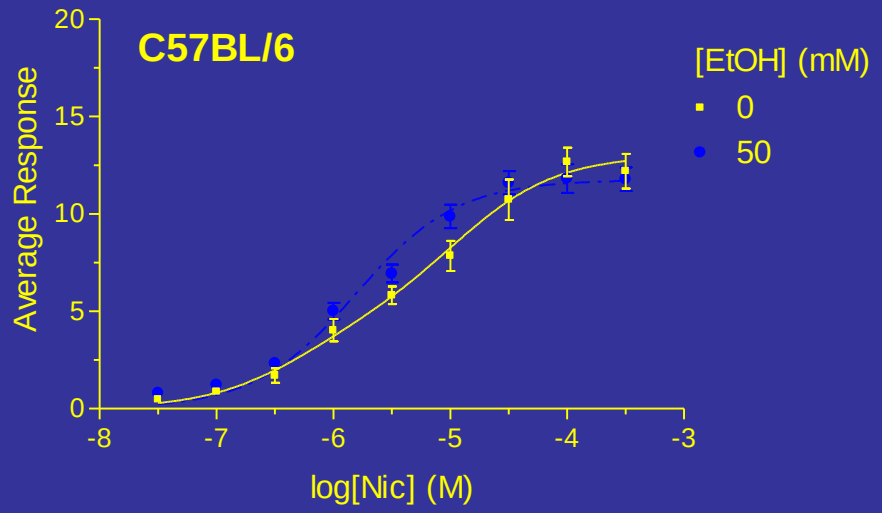
Cardoso *et al*. (1999)

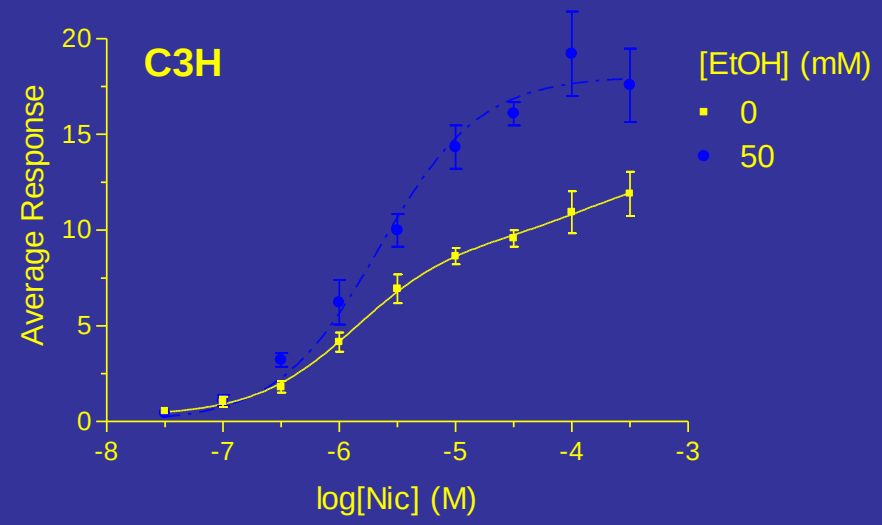
JPET 289: 774-780.



Does the A/T polymorphism Influence the Effects of Ethanol on Receptor Function?

Strain Differences in EtOH Effects on $^{86}Rb^{+}$ flux





Does the α4 A/T polymorphism influence behavioral effects of nicotine and ethanol?

Acoustic Startle Apparatus

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

Associations Between A/T Poly and Acoustic Startle

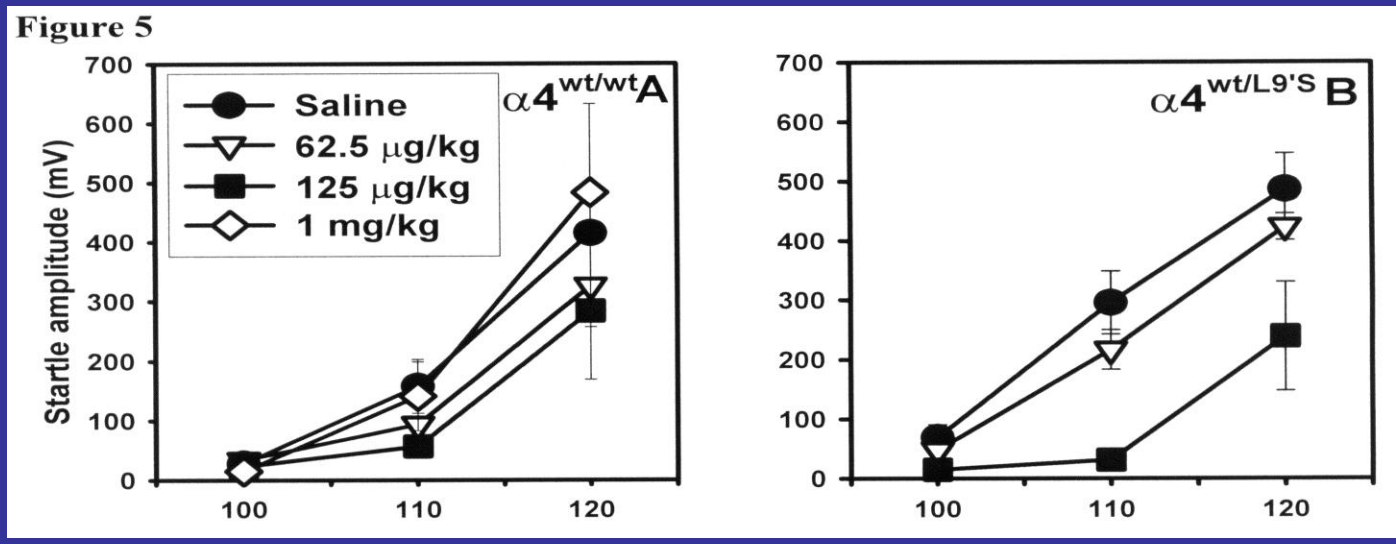
- Nicotine-induced INCREASES in startle are associated with the “poly” in inbred strains.
- Nicotine-induced INCREASES in startle are associated with the “poly” in LS-SS & LS-x-SS RI strains.
- Alcohol-induced DECREASES in startle are associated with the “poly” in LS-SS & LS-x-SS RI strains.

Reverse Genetics Provides Converging Evidence

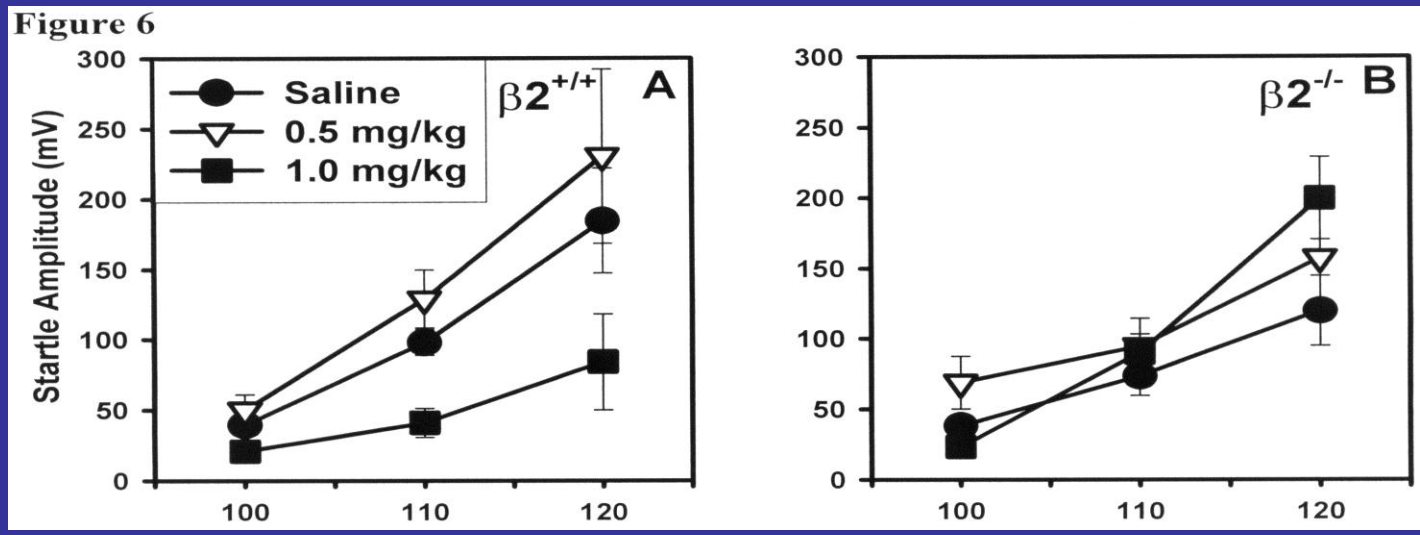
- Studies with null mutants
  - α4 mutants (John Drago, Melbourne)
  - β2 mutants (Marina Picciotto, Yale)
  - Others (Beaudet, Baylor; Heinemann, Salk)
- Studies with gain of function mutants
  - Gain of function α4 mutants (Lester, Cal Tech)

Nicotine effects on Startle in α4 and β2 mice

- α4 L9'S Hets are more sensitive to the effects of nicotine



- β2 mutants are less sensitive to the effects of nicotine



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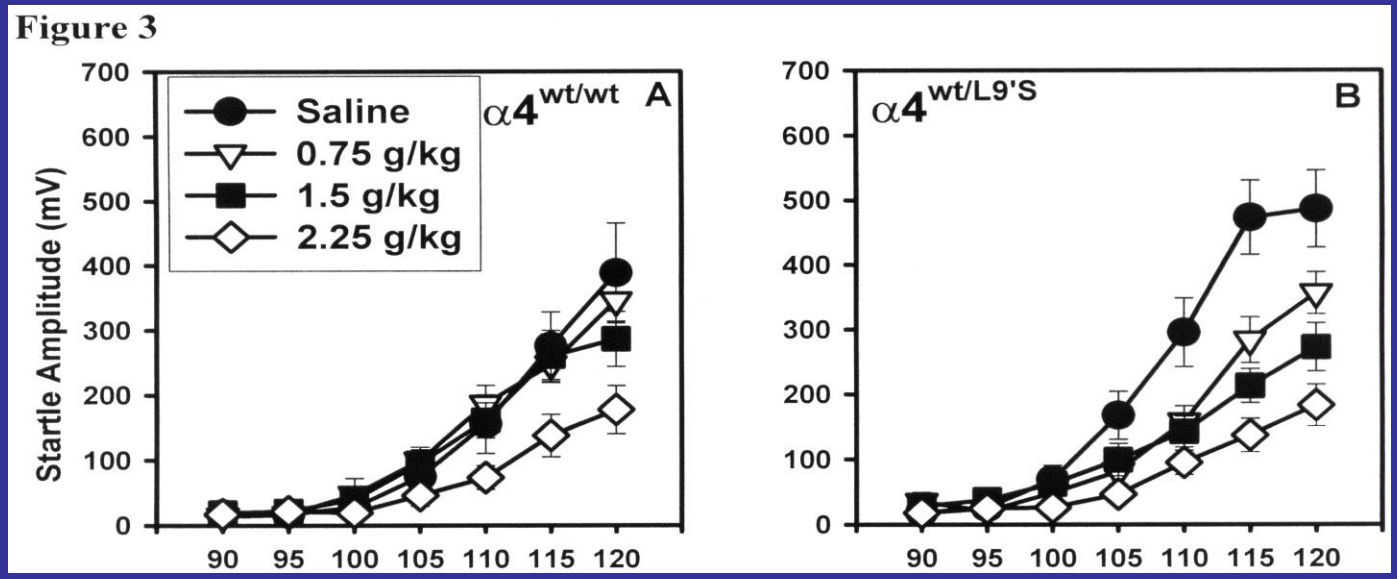

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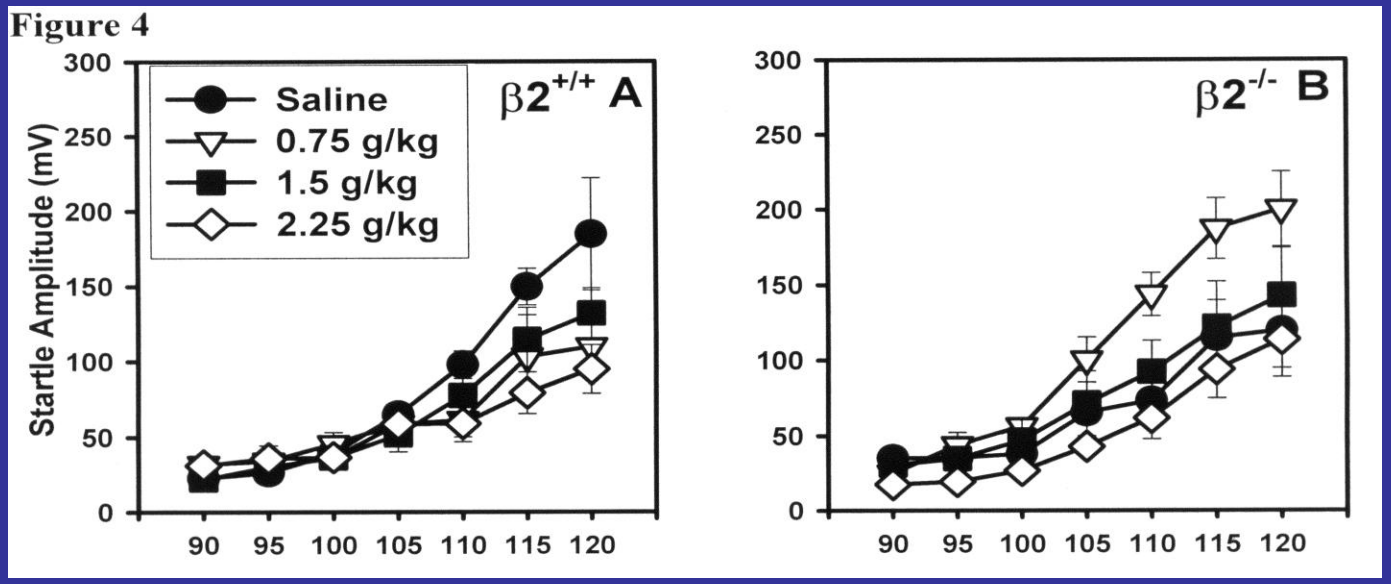


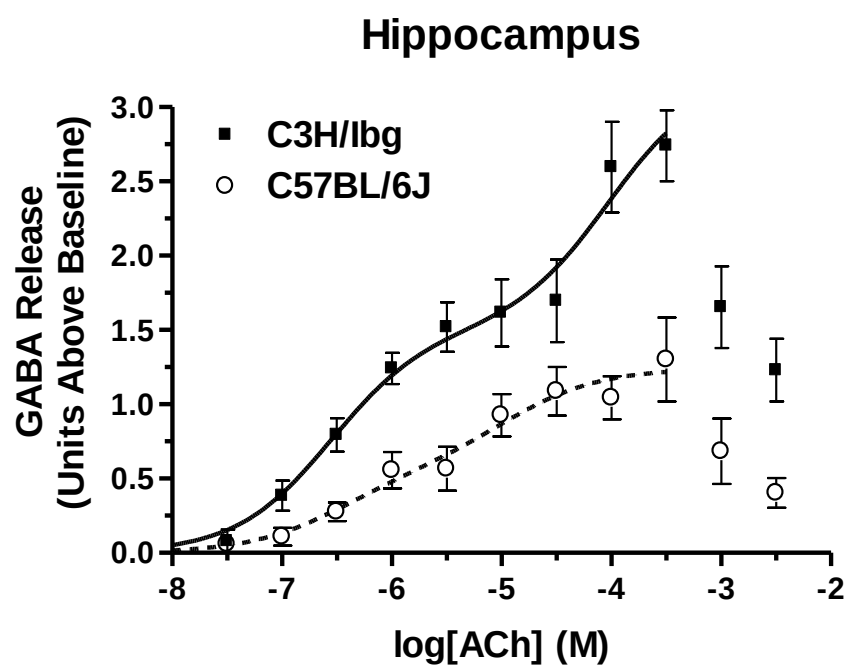
SUMMARY

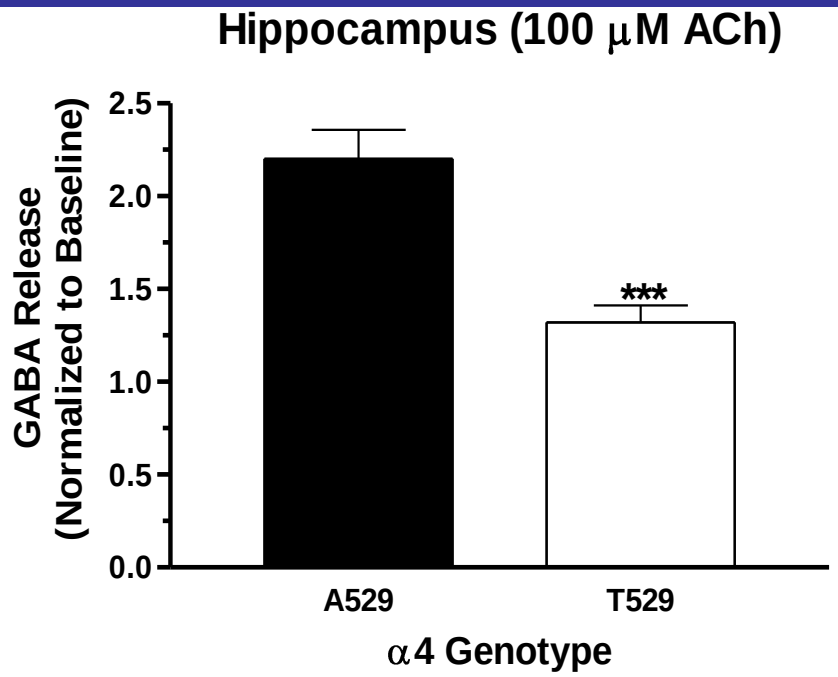
- The A529T α4 polymorphism results in alterations in receptor function, measured in vitro.
- The A529T α4 polymorphism affects sensitivity of the receptor to ethanol, measured in vitro.
- The A529T α4 polymorphism is associated with variation in SOME, particularly “excitability” measures, responses to alcohol and nicotine.

Localization and function of α4-containing receptors

- Expressed throughout the brain almost invariably with β2.
- Most are presynaptically expressed where they modulate neurotransmitter release
  - Dopamine
  - GABA
  - More?

Mouse Strains Differ in GABA Release





The Withdrawal Model

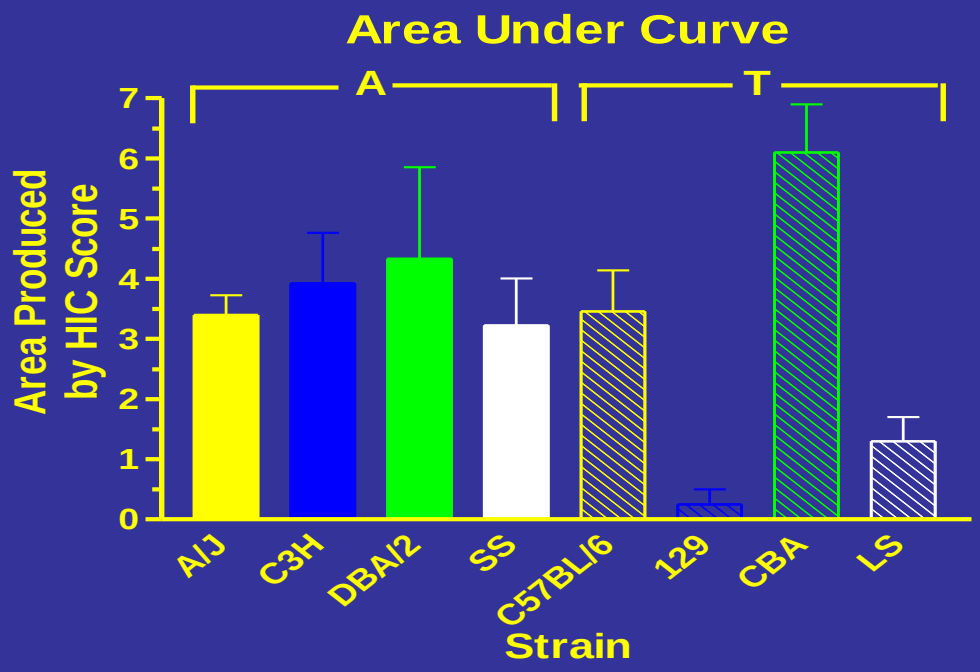
- Chronic drug use results in changes in brain chemistry and function that are “opposite in nature” to the acute effects produced by the drug.
- Behavioral signs associated with drug cessation are “uncomfortable” and often are opposite of those produced by the drug.
- Avoiding withdrawal “sickness” drives further drug use.

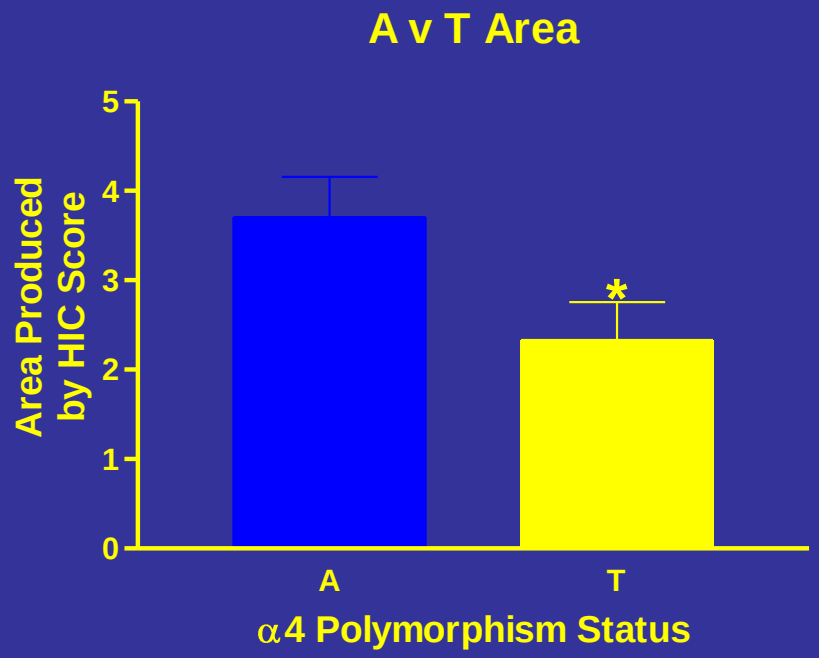
Common Features of Alcohol and Nicotine Withdrawal

- Hyperexcitability (tremors, convulsions).
- Increased anxiety.
- Decreased cognitive function.
- Altered HPA axis.
- More……

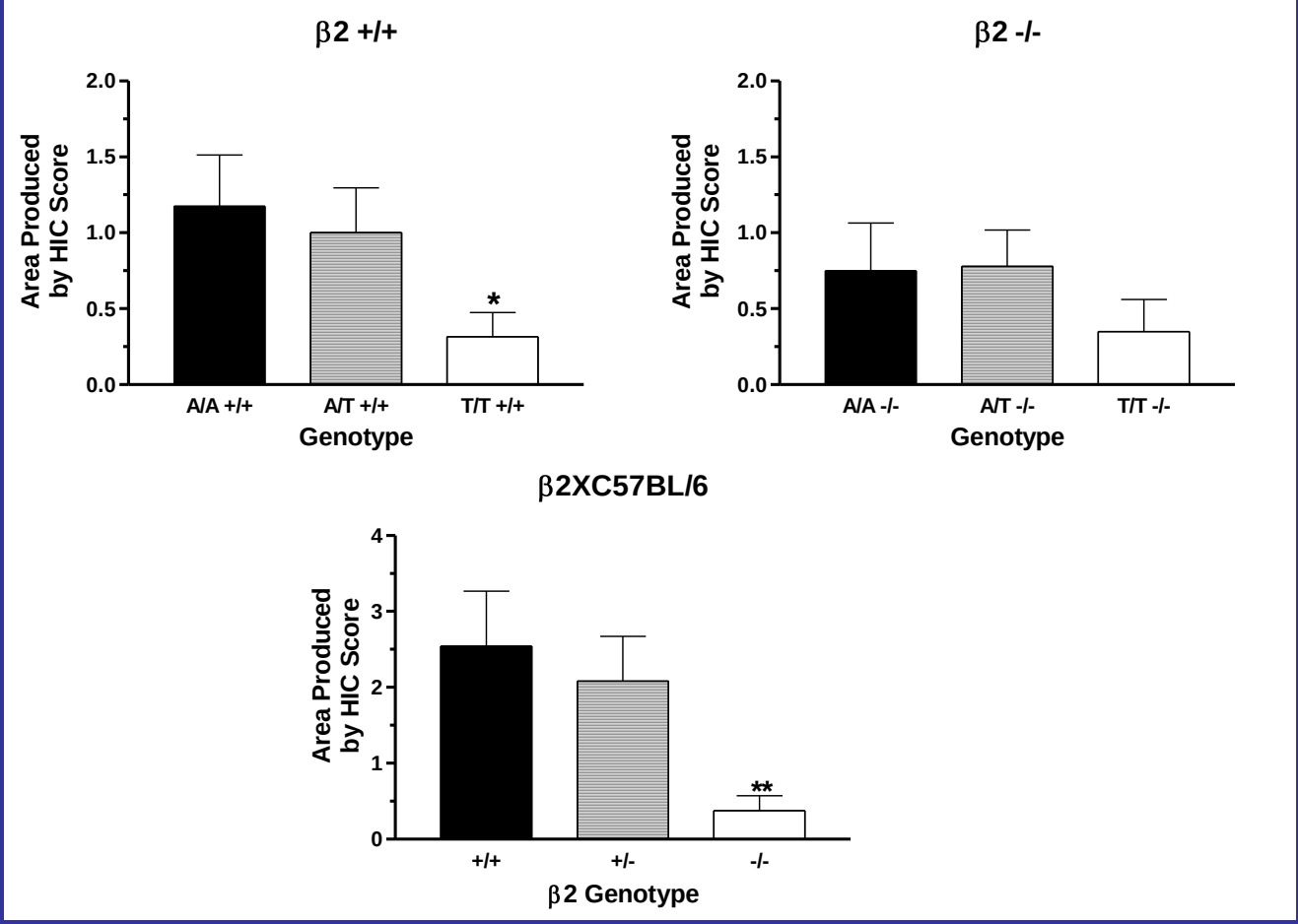
We Have Studied Withdrawal Following a Single, High Dose of Alcohol (hangover) Using Handling-Induced Convulsions as a Convenient Measure.

α4 A529T and Ethanol-induced HIC





The A/T polymorphism and Chrnb2 play a significant role in the severity of EtOH withdrawal



Butt *et al.* (2004) *JPET* 308: 591-99

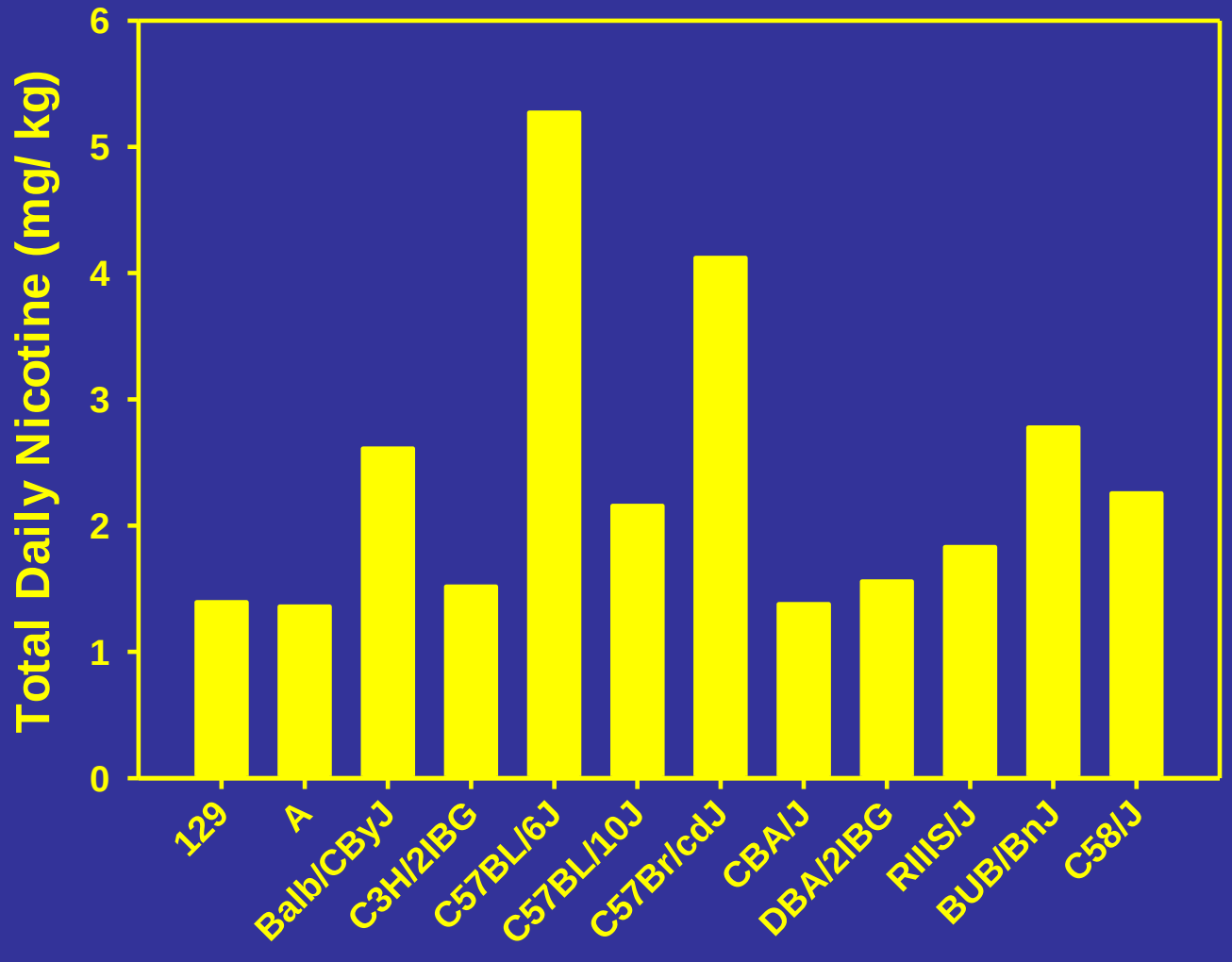
The Reinforcement Model

- Many view drug reinforcement as THE MOST IMPORTANT component of addiction.
- All drugs that release dopamine are self-administered by animals and man.
- Drugs that block DA receptors decrease self-administration.
- Drugs that block DA receptors ARE NOT effective in treating addiction to ANY drug.

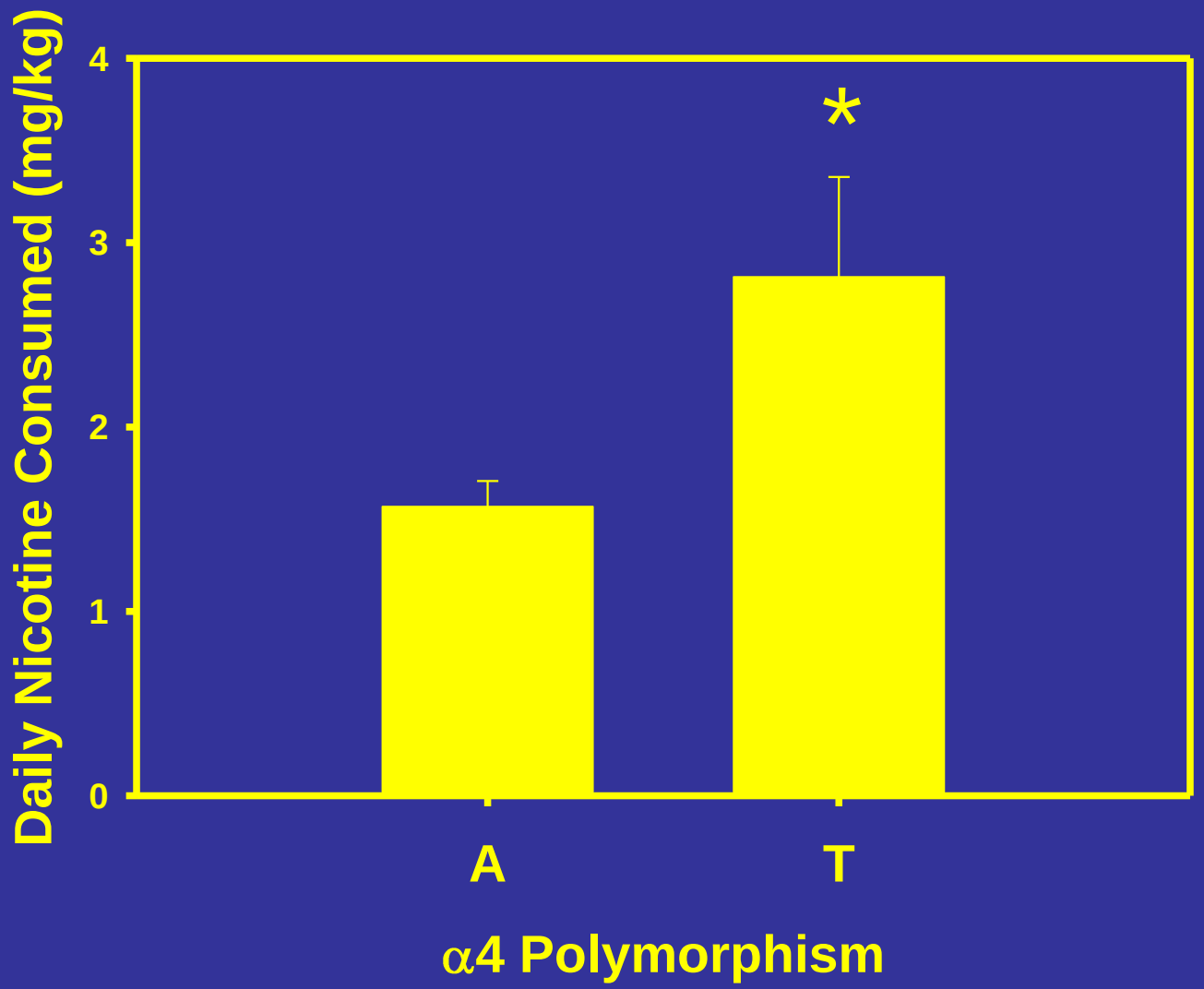
How do we measure reinforcing effects of Alcohol & Nicotine?

- i.v. self-administration (nicotine).
- Operant responding for oral ingestion (alcohol).
- Conditioned Place Preference (nicotine and alcohol).
- Oral Preference (Nicotine and Ethanol).

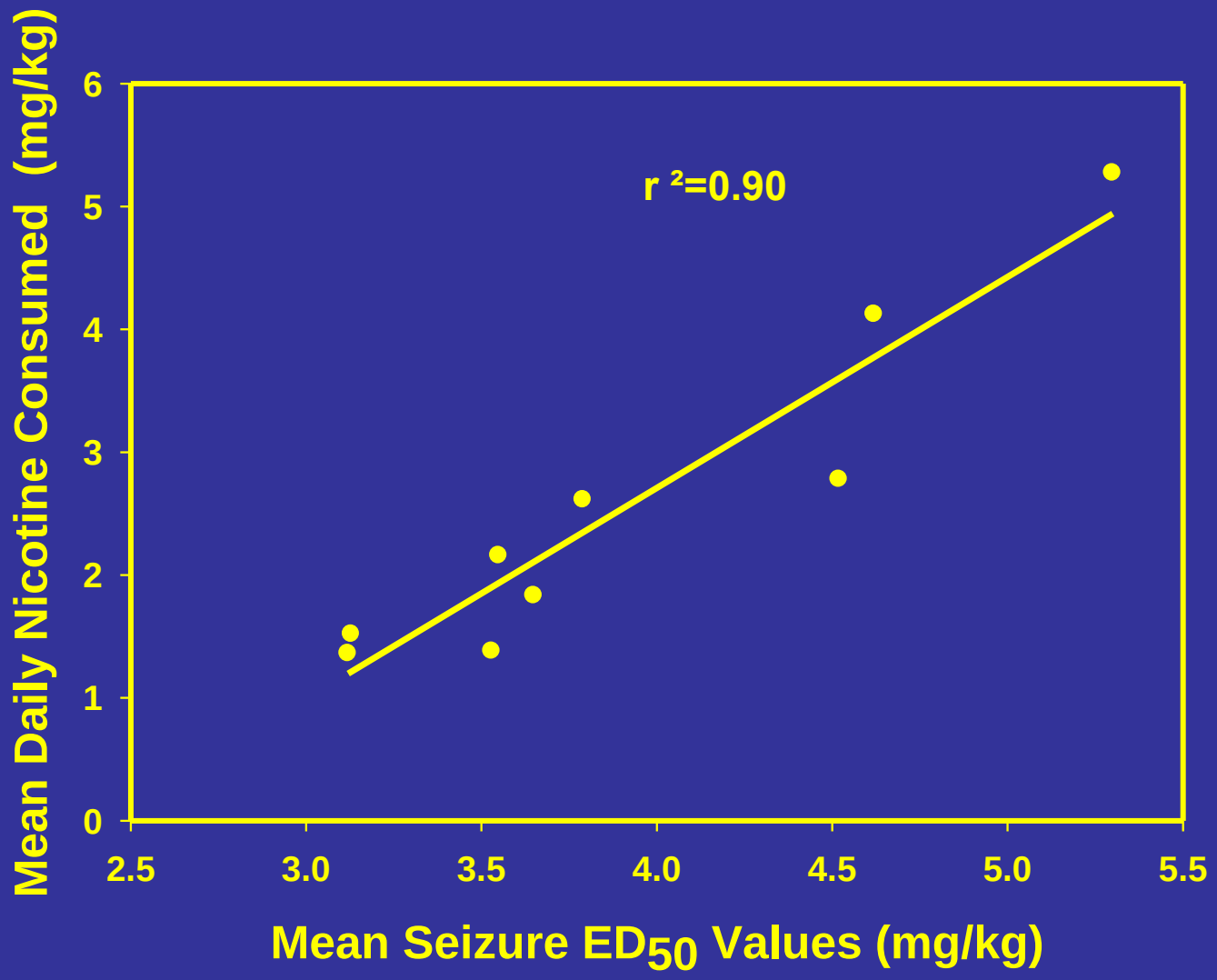
Total Nicotine Consumption by Strain



Effect of the A/T α4-Polymorphism on Nicotine Consumption

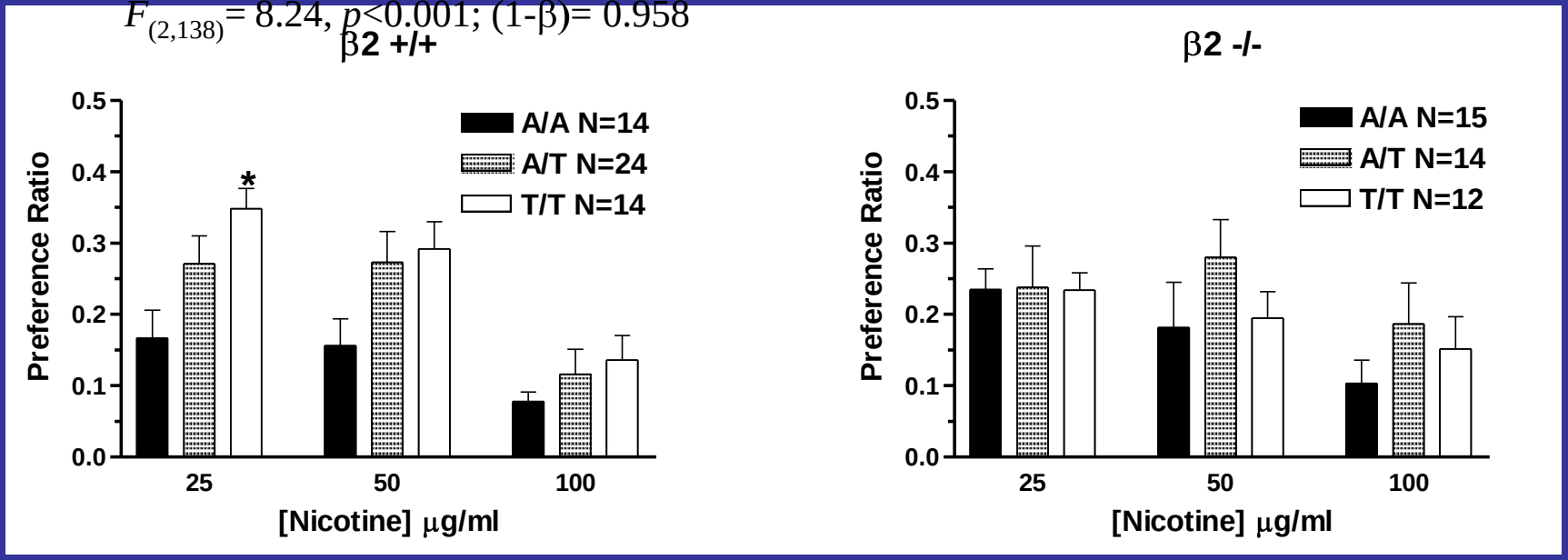


Correlation Between Nicotine Consumption and Seizure Sensitivity



Nicotine preference is modulated by the A/T polymorphism

$F_{(2,138)} = 8.24$, $p<0.001$; $(1-\beta)= 0.958$



Butt *et al*. (2004) *Behav. Neurosci*. In press.

Alcohol Preference IS NOT Influenced by the α4 A/T Polymorphism (sigh!)

The α4 A/T Poly Influences

- nAChR receptor function.
- EtOH enhancement of receptor function.
- EtOH effects on receptor desensitization.
- Sensitivity to several effects of nicotine.
- Sensitivity to several effects of alcohol.
- The development of tolerance and cross tolerance between nicotine & alcohol.
- Severity of alcohol withdrawal.
- Nicotine preference.
- More…..

Problems with the Pharmacological Model of Addiction

- Despite intensive investigation this model has not led to novel treatments for addiction.
- Pharmacological model studies have not identified genes that have been verified in humans.
- Model does not account for craving and the role that secondary reinforcers play in modulating continued use and abuse.

We’re Just At The Starting Line

THANKS

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