Pharmacogenetic Approaches to the Treatment of Alcoholism: Preclinical Studies

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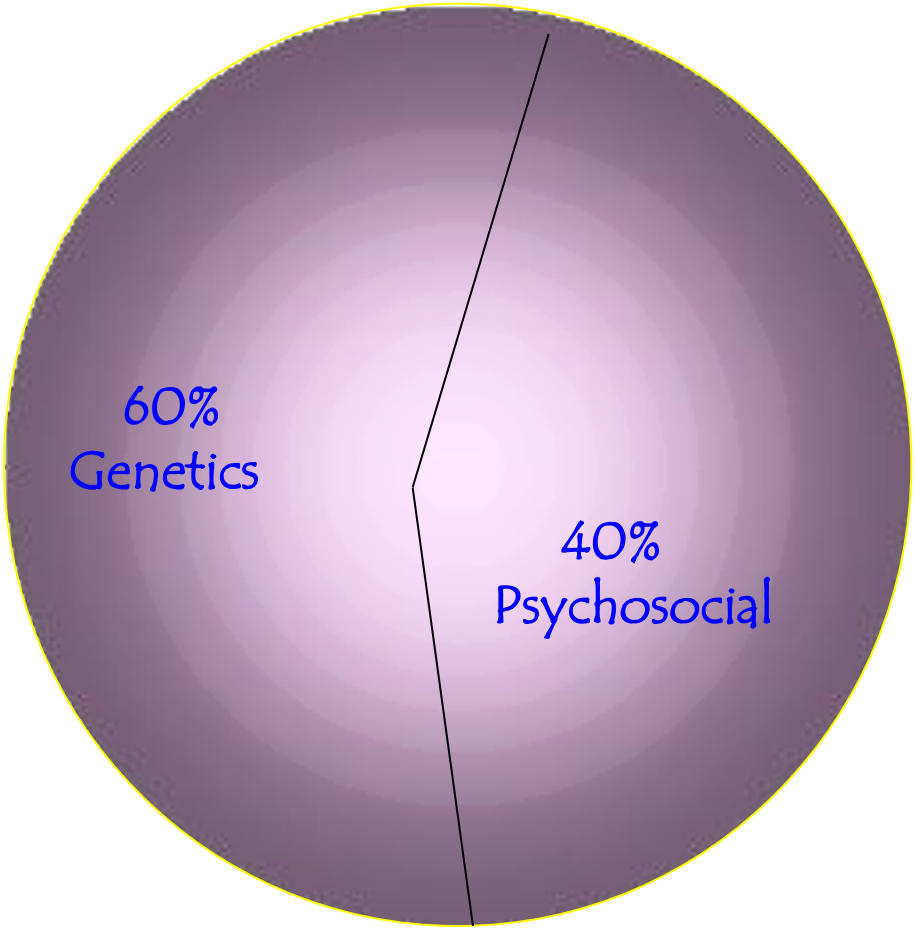
Factors that influence the Probability of Developing Alcoholism

Identical vs Fraternal twins

Children adopted at an early age.

SNPs in the genome of affected families

Genetic protection in Asians: AVERSION



Price

Availability

Laws

Permissiveness

Recognition of Risk

ONCE ALCOHOLISM HAS DEVELOPED it is maintained by:

Positive Reinforcement
(rewarding effects and no aversive effects)

Negative reinforcement
(reducing withdrawal symptomatology)

Conditioning (situational)
(memory, stress: "Craving")

TREATMENT DEALS MOSTLY WITH:

Positive Reinforcement
(reduce rewarding effects; increase aversive effects)

Negative reinforcement
(reducing withdrawal symptomatology)

Conditioning (situational)
(memory, stress: "Craving")

ANIMAL MODELS FOR ALCOHOLISM THERAPIES

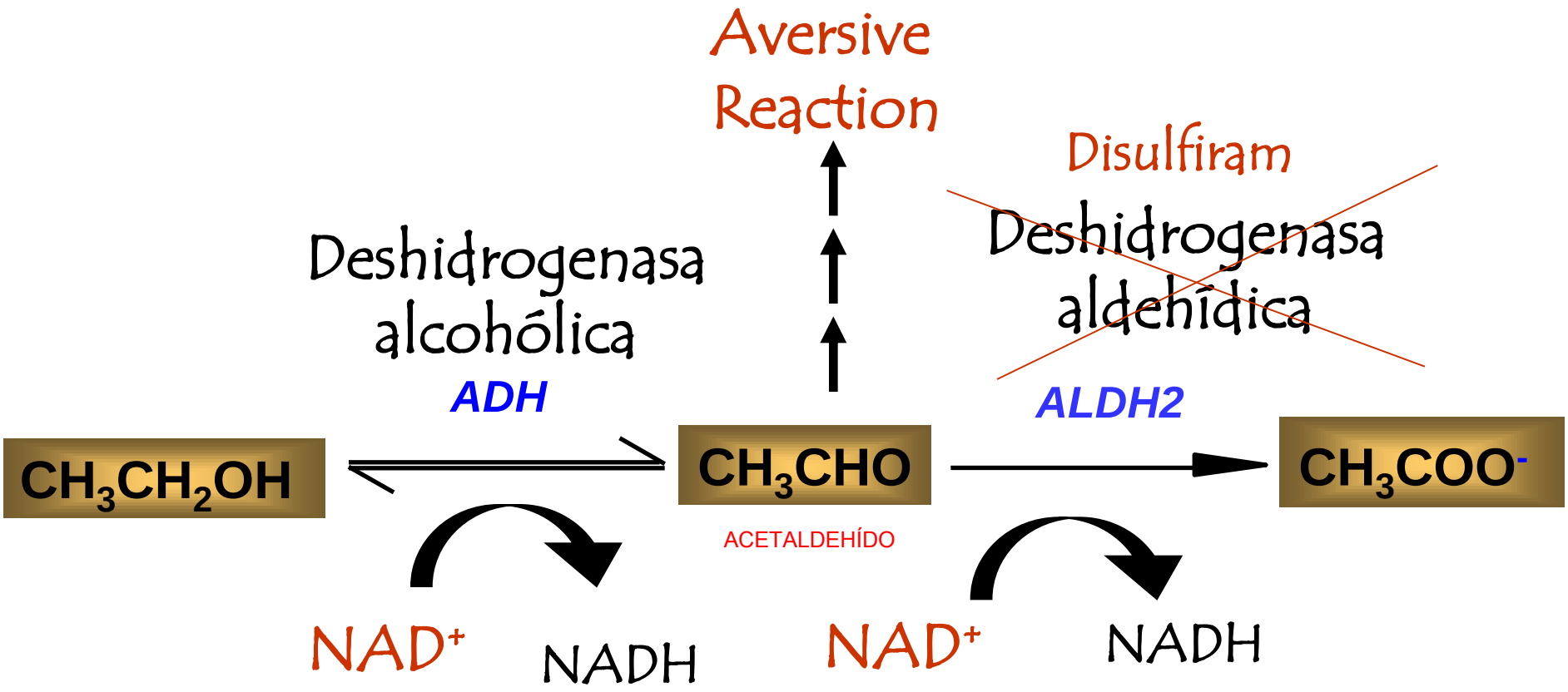
Positive Reinforcement

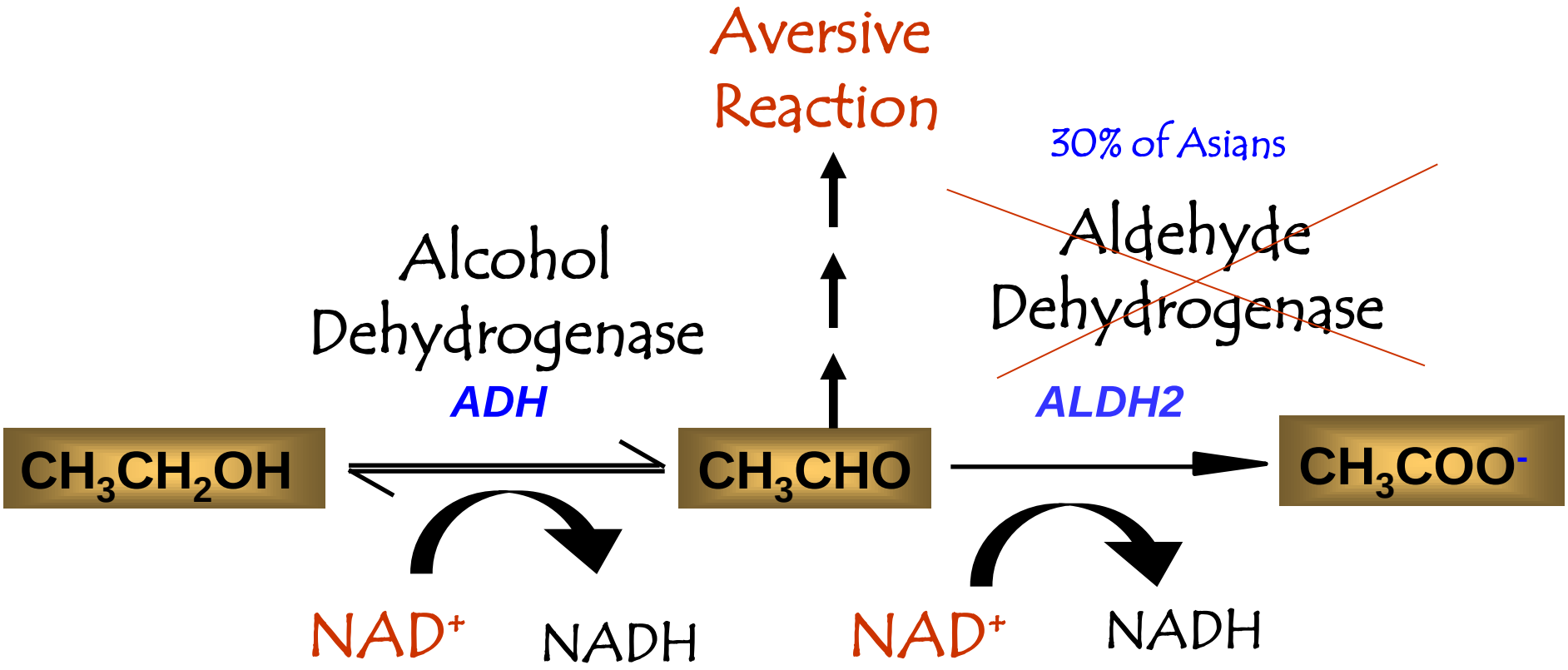
(increase aversive effects: reduce rewarding effects)

Conditioning (situational)

(Memory, stress, psychotherapy)

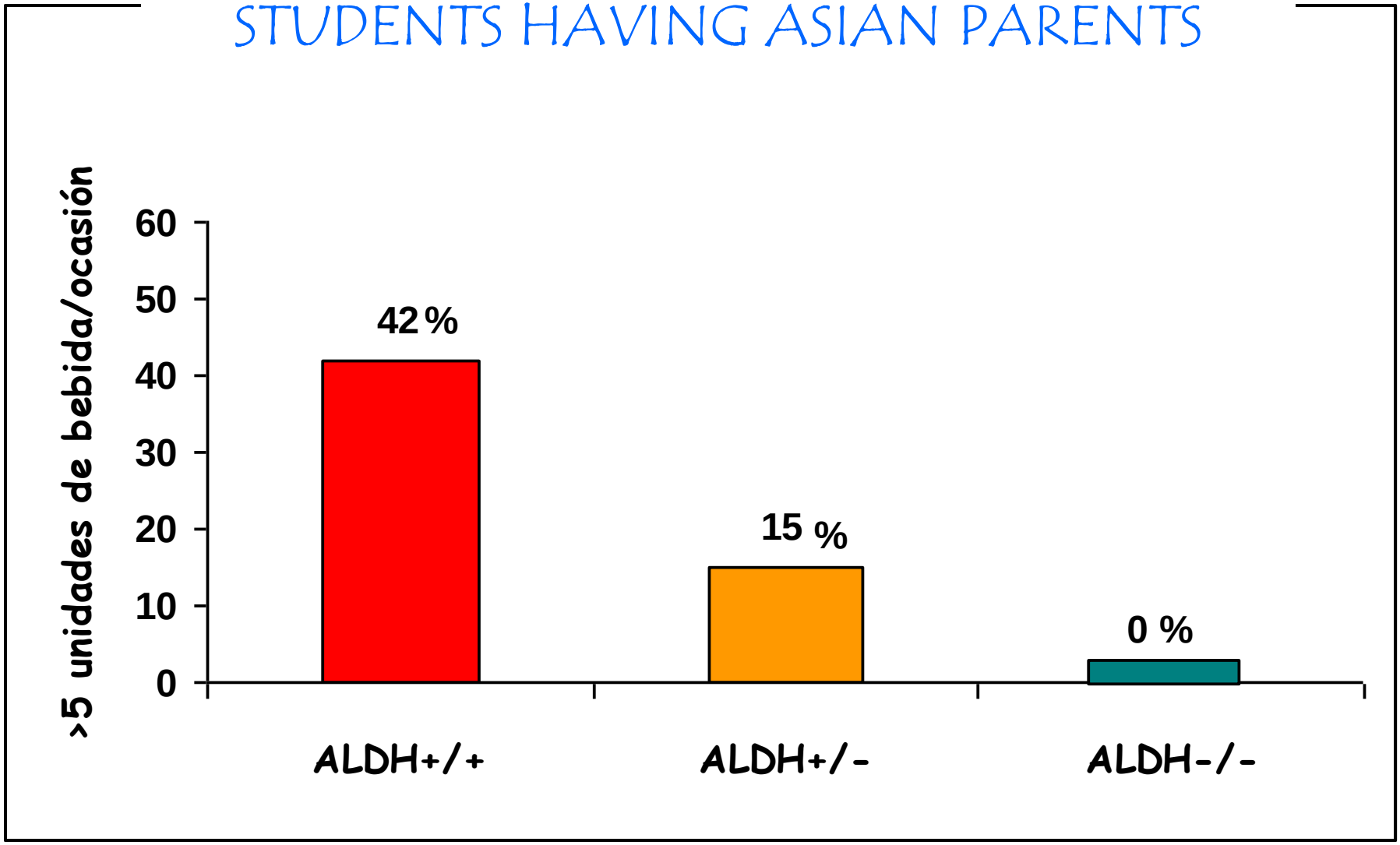
Metabolism of Alcohol





NATURE's DISULFIRAM:
Inactivating mutation of ALDH2: an effect analogous to that of disulfiram
Reduction of Alcoholism prevalence inAsia:
ALDH2 +/- = - 67%
ALDH2 -/- = - 99%

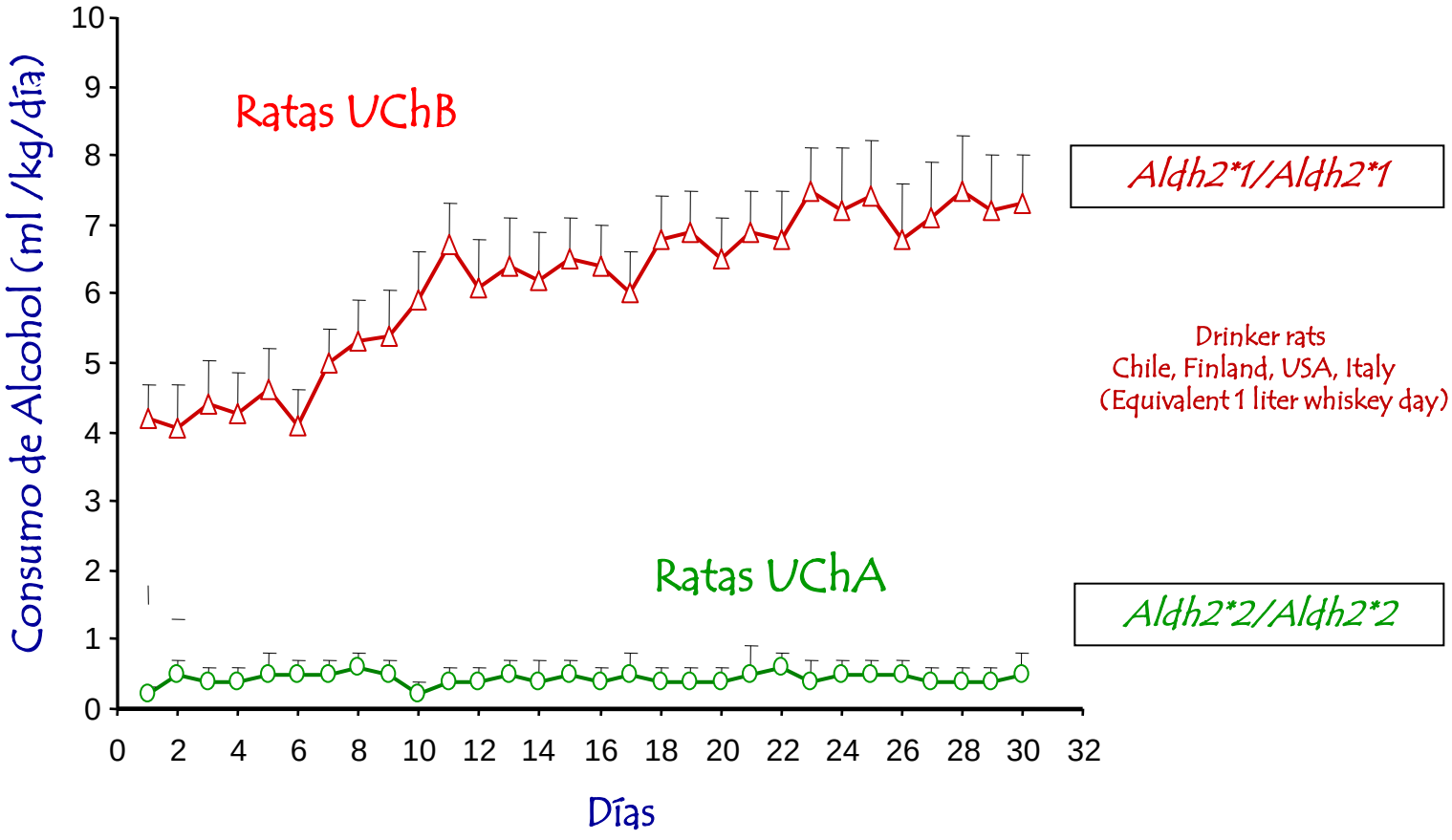
ALCOHOL ABUSE IN NORTH AMERICAN STUDENTS HAVING ASIAN PARENTS



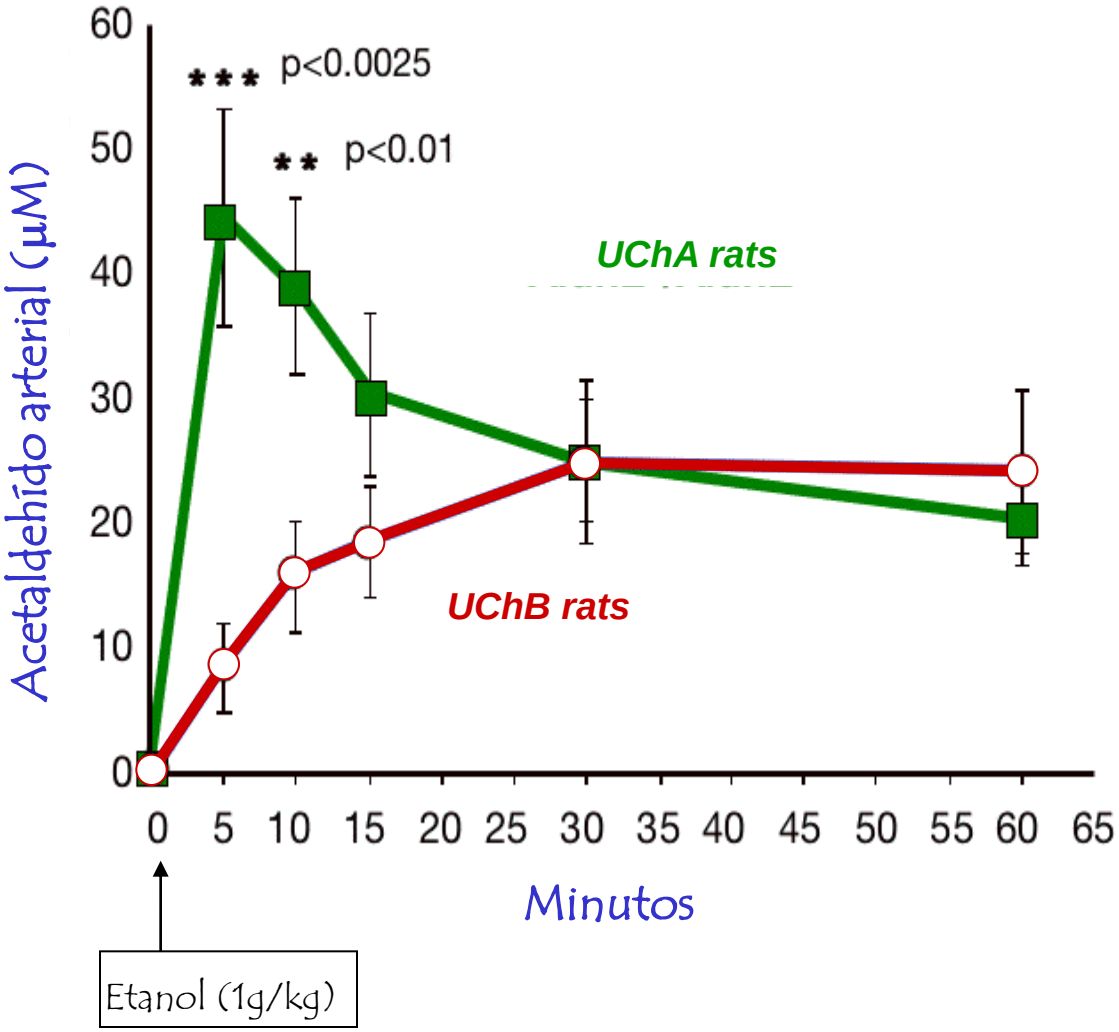
Selective breeding of RATS :

UChA (abstainers) y UChB (bibulous)

Alcohol (10%) and water available 24 hours/day



ABSTAINER RATS ALSO SHOW ELEVATIONS IN BLOOD ACETALDEHYDE LEVELS WHEN ADMINISTERED ETHANOL



Antisense molecules bind to the mRNA like a magnet, blocking the gene message

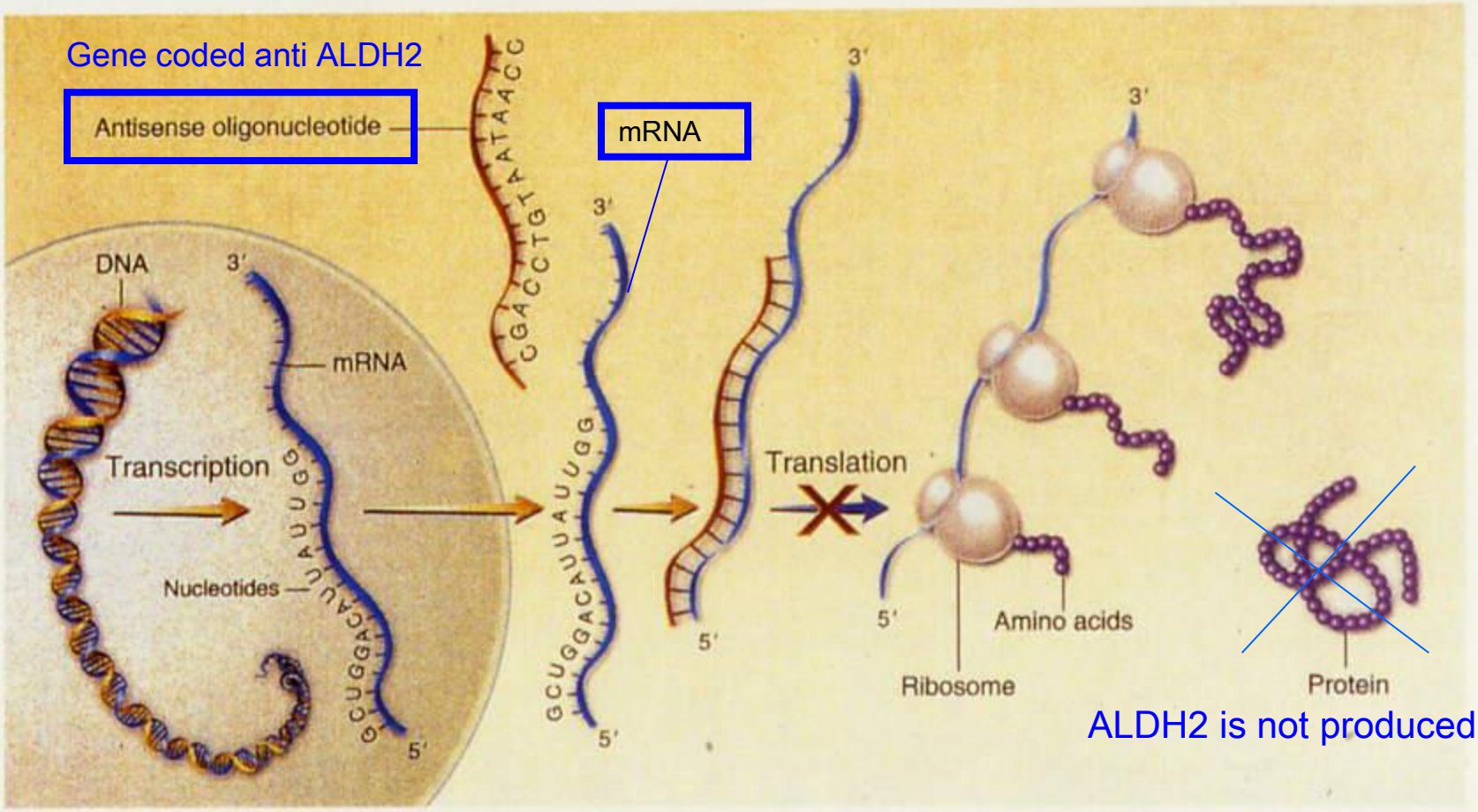
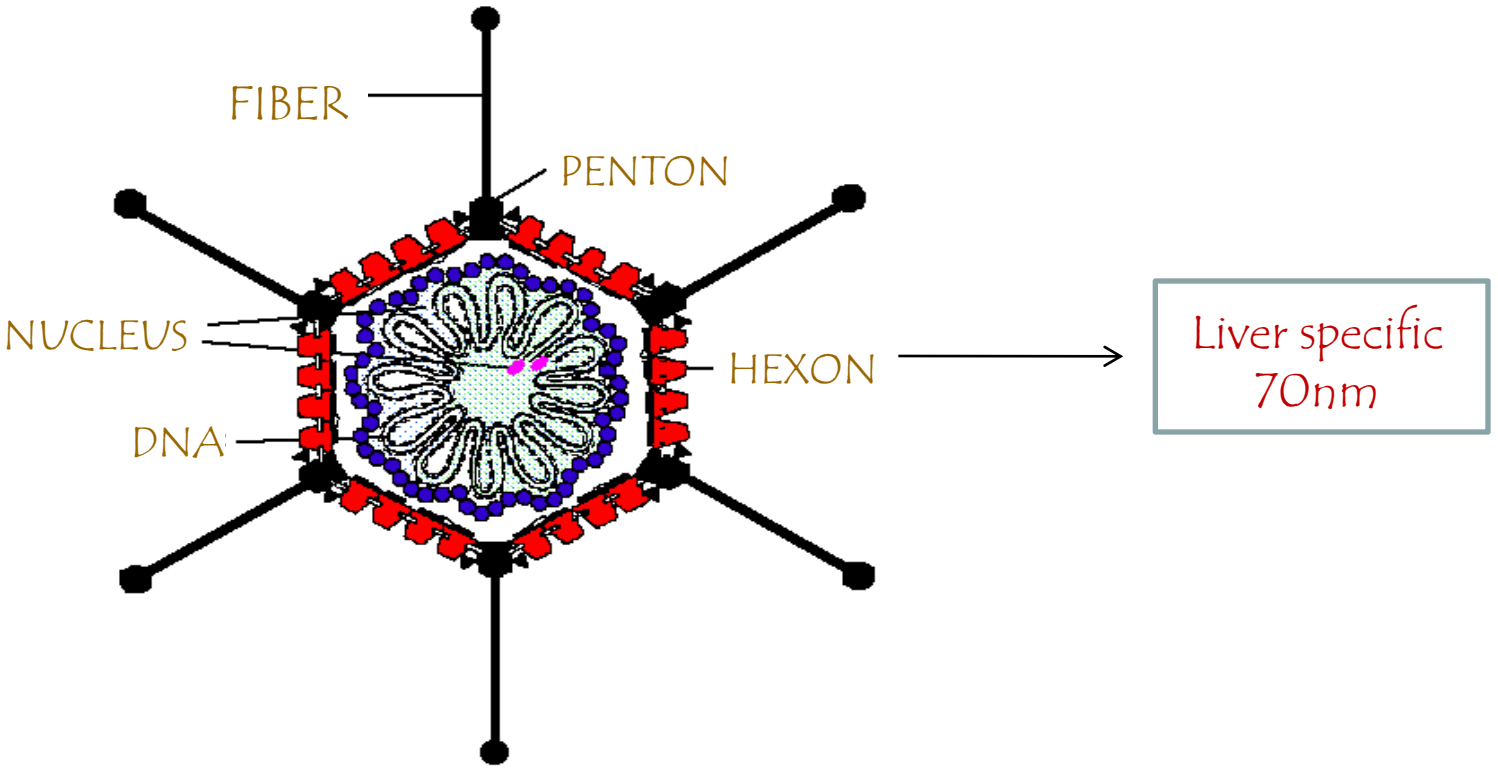
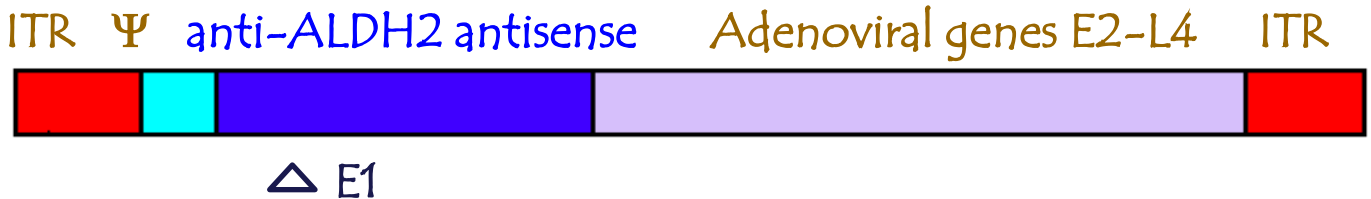


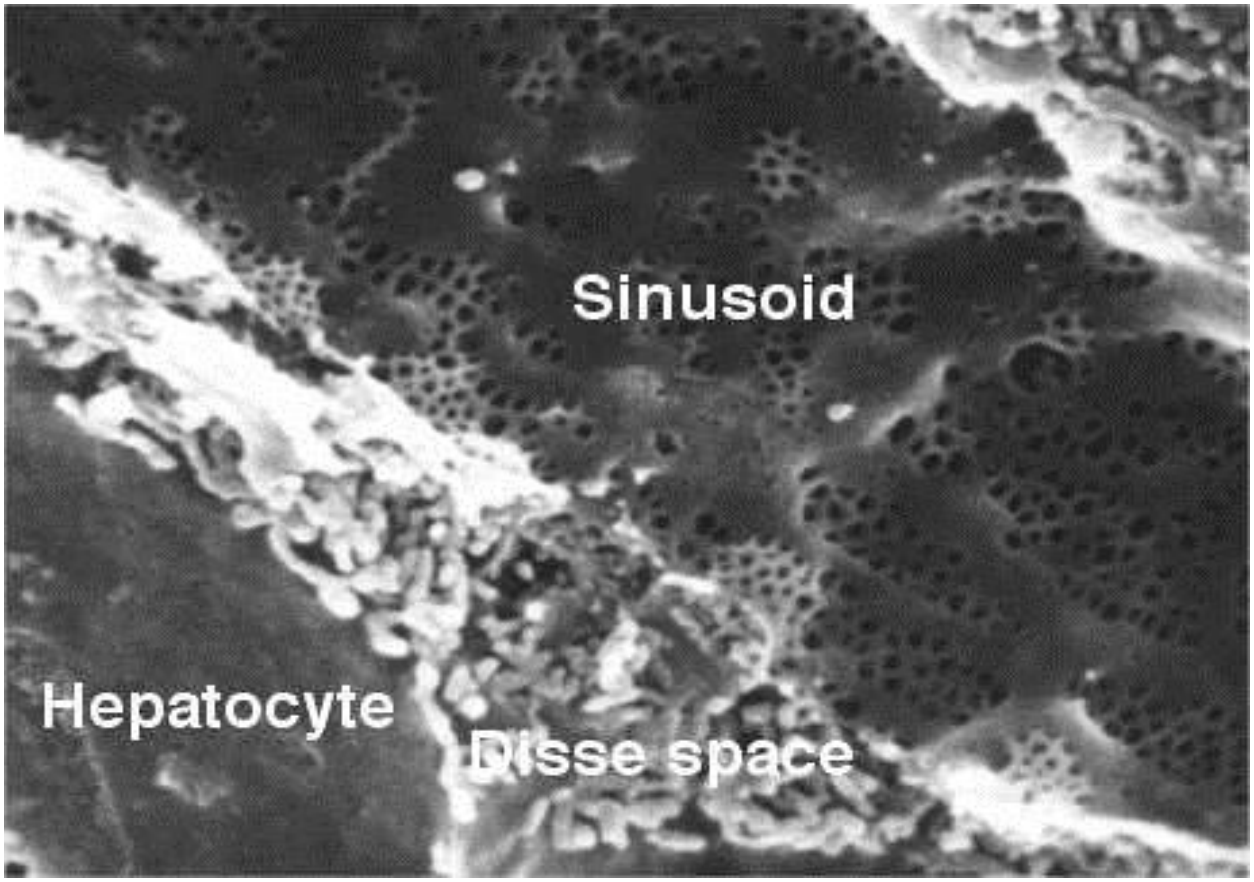
FIG. 1. A classical antisense strategy using complementary DNA. Transcription of DNA generates an mRNA (which would be far longer than the molecule depicted here). A short complementary DNA molecule, the antisense ODN, binds to the mRNA, forming a DNA-RNA hybrid region stabilized by Watson-Crick base pairs (G-C and A-U/A-T). Once the hybrid region has formed, the mRNA is no longer functional. Although a typical mRNA contains about 1,500 nucleotides and thus extends beyond the hybrid region, the antisense DNA renders the entire mRNA unsuitable for translation into protein. Thus, protein synthesis is prevented. Reprinted from *The New England Journal of Medicine*,[5] with permission of the publisher and Dr. W. Michael McDonnell (University of Michigan).

Generation of an Adenoviral Vector carrying an anti-ALDH2 antisense gene (Troyan Horse)





Preferential entry of Adenoviral vectors into liver cells (hepatocytes)

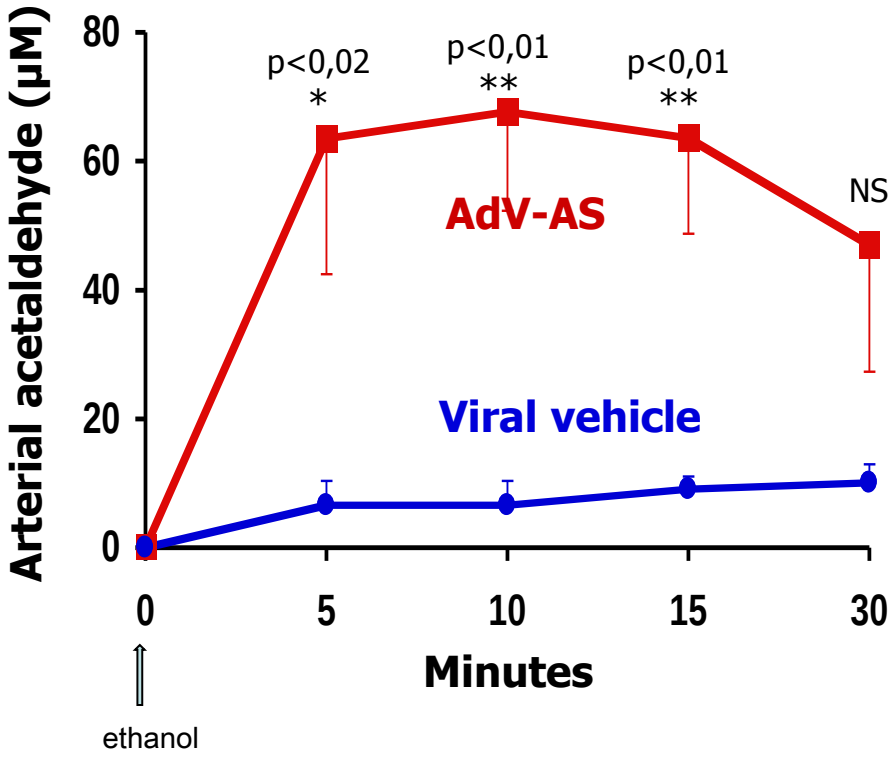


Fenestra: (pores) 300 – 1000 nm. Adenovirus: 70 nm ; Other capillaries: <20nM

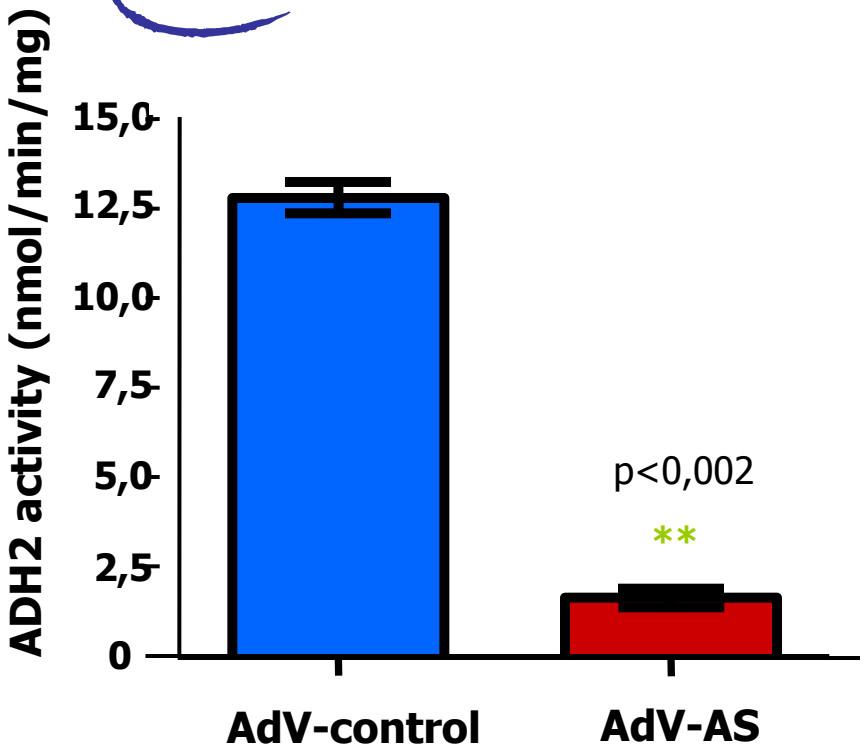
Micrograph of Robin Fraser, University of Otago, New Zealand)

Effect of i.v antisense gene anti ALDH2 on ALDH2 activity and blood acetaldehyde

Arterial acetaldehyde following Ethanol 1g/kg i.p.

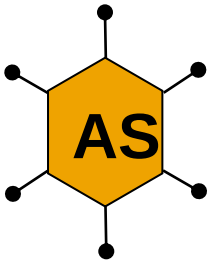


Liver ALDH2 activity



UChB rats allowed alcohol consumption for two months

Antisense anti ALDH2 generated by adenovirus (AdV-cDNA ALDH2-AS)



AdV-*Aldh2*-AS

2 months

Free access to alcohol (10%) and water

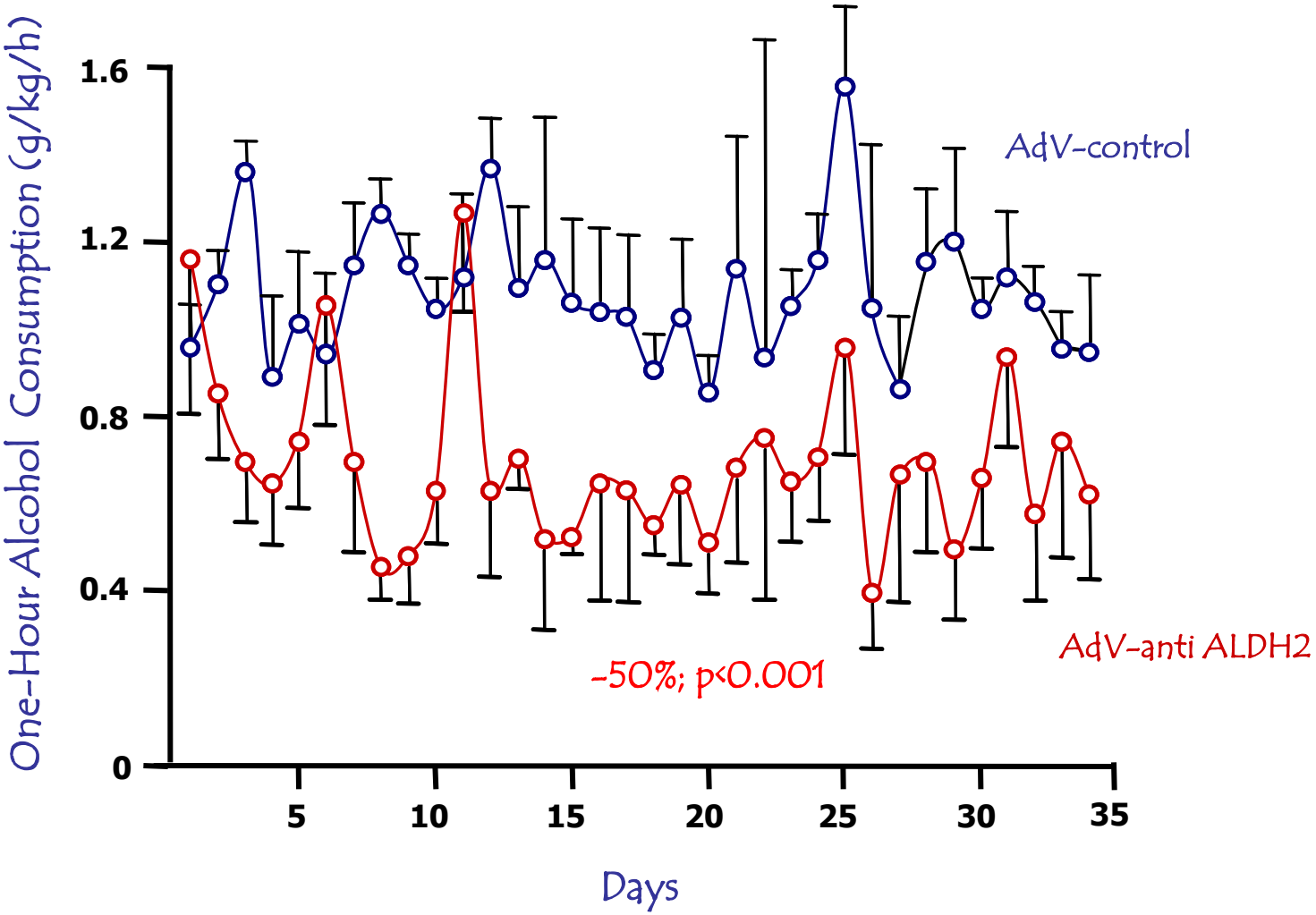
3 días

Without alcohol

35 days

Happy hour 10% alcohol and water

Proof of Principle:
Happy-hour by alcohol-dependent UChB rats
(after 2 months of ethanol free choice)

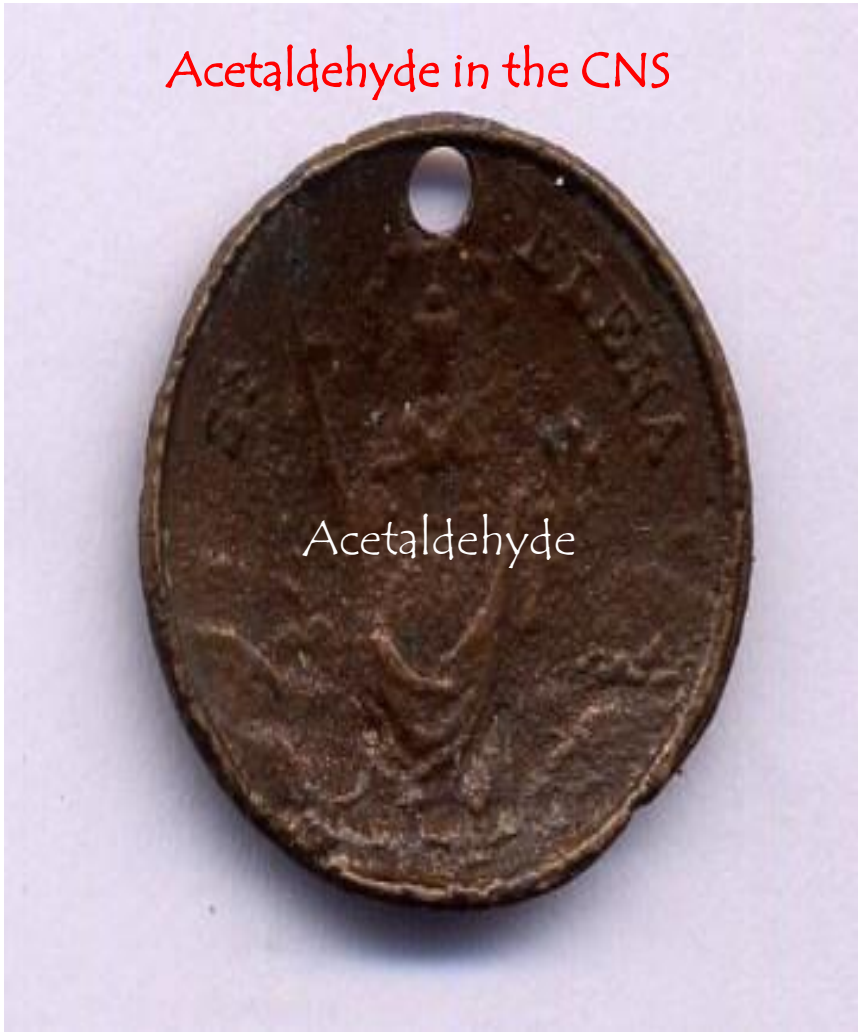


Acetaldehyde : A NEW FRONTIER

Reward:

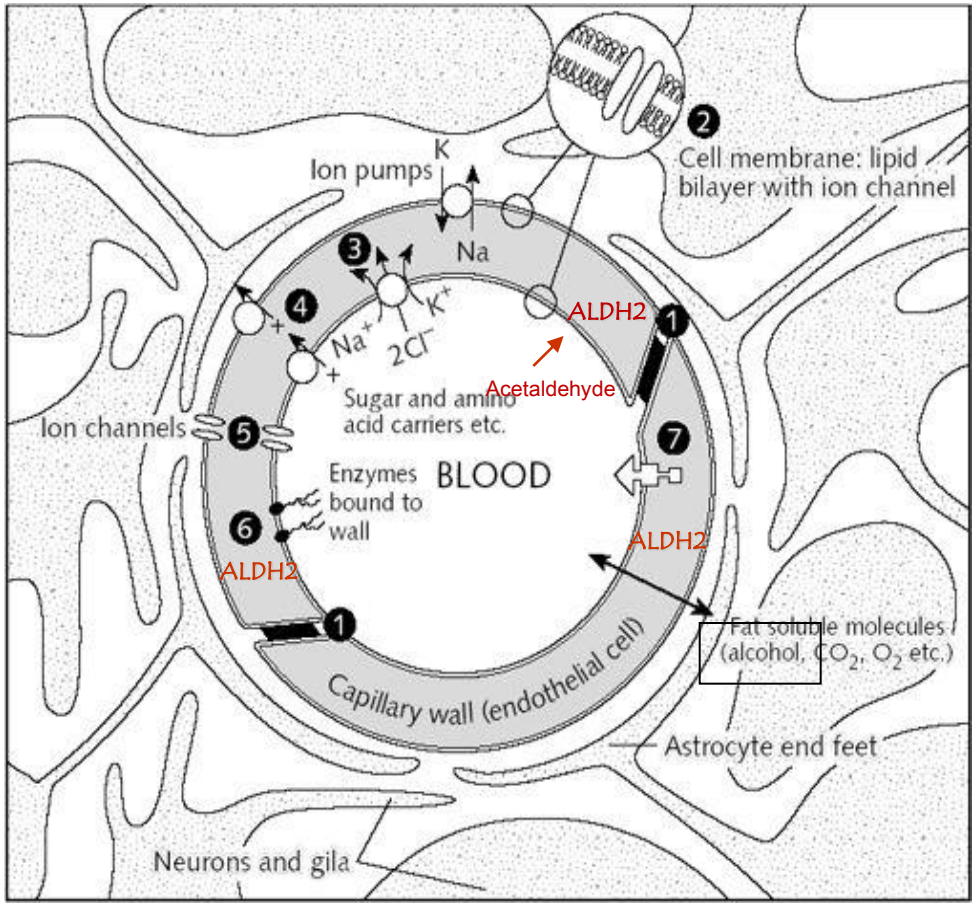
Acetaldehyde produced in the liver

Acetaldehyde in the CNS



Aversion:

Does Acetaldehyde cross the blood-brain barrier?



NO:

At the levels of acetaldehyde present in blood, the enzyme ALDH2 in the tight-junction endothelial cells of brain capillaries oxidizes acetaldehyde into acetate

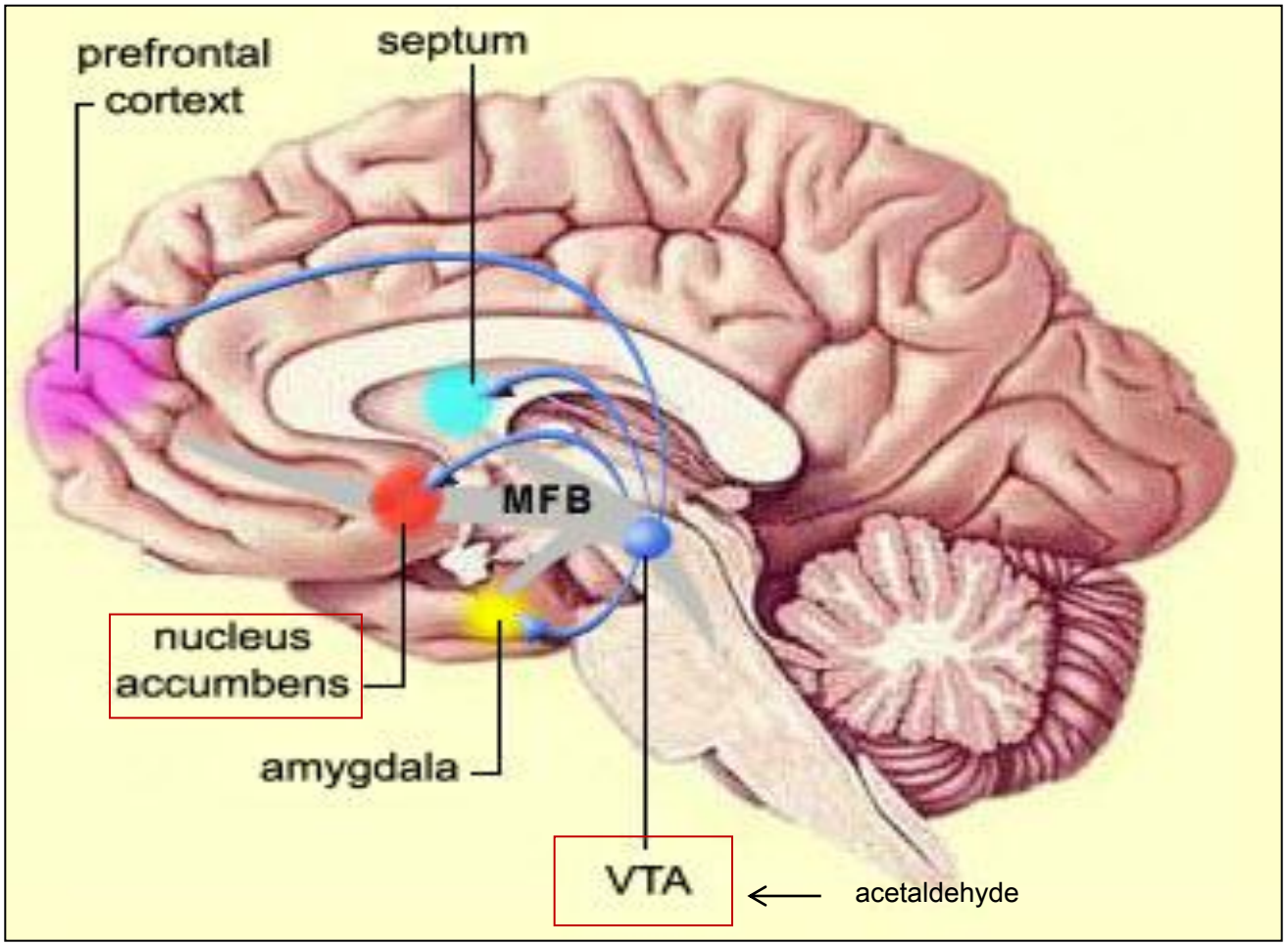
Acetaldehyde : A NEW FRONTIER

Reward:

Acetaldehyde produced in the liver

Acetaldehyde in the CNS

Aversion:



The dopaminergic neurons in the ventral tegmental area project neuronal axons into the nucleus accumbens releasing dopamine

The other side of the Coin

Recent literature indicates that
in the brain acetaldehyde is not aversive but rather reinforcing

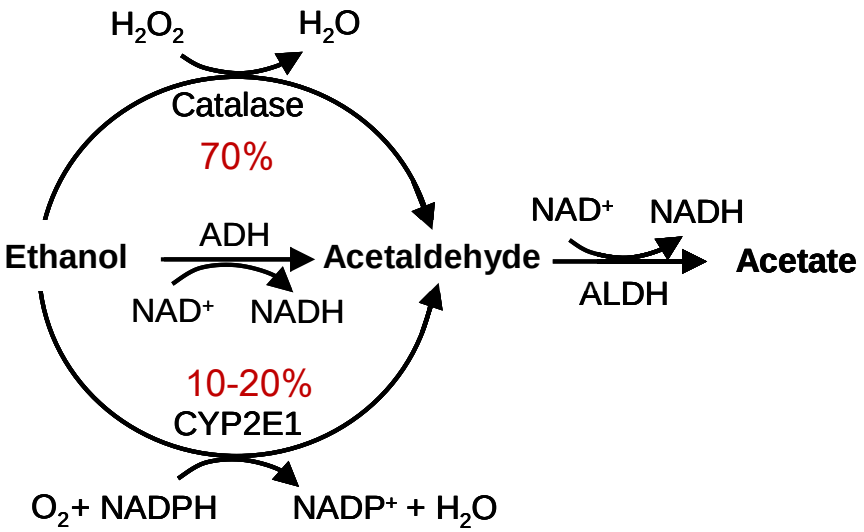
Rat bred as alcohol drinkers (Indianapolis P rats) will self-administer acetaldehyde into the dopaminergic neurones of the ventral tegmental area of the brain (VTA):

(Concentration needed to promote self administration)

Ethanol	0.02 M
Acetaldehyde	0.00005 M

Rodd et al, Alcoholism Clinical and Experimental Research 2005; 2008

Is ethanol metabolized into acetaldehyde in the brain?



YES

Although there is no alcohol dehydrogenase in the brain, catalasa and CYP2E1 present in the brain are able to metabolize ethanol.

There is also an active brain aldehyde dehydrogenase .

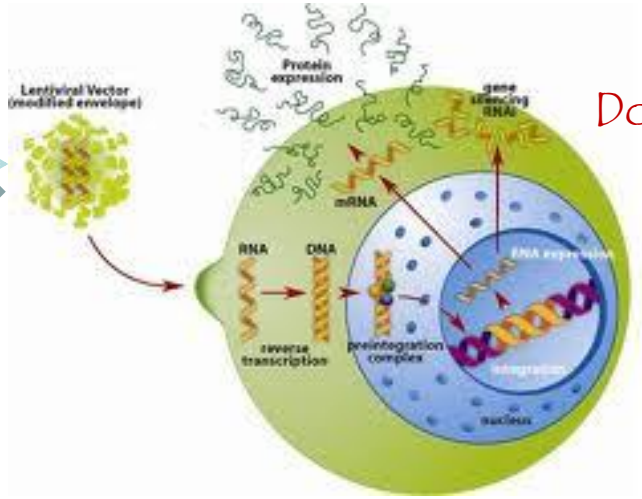
Then lets

(a) lower the activity of catalase or

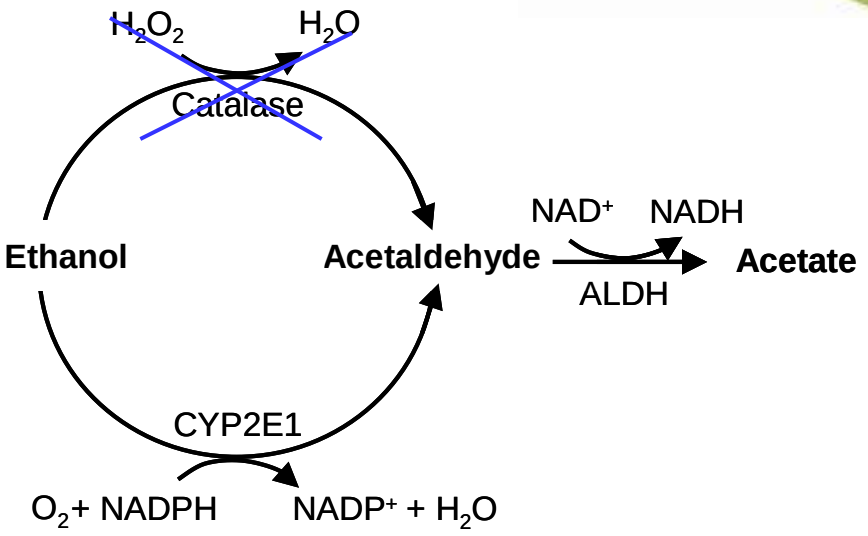
(b) increase activity of aldehyde dehydrogenase

Injection of Lentiviral Vector (gutless)

shRNA anticatalase Lenti

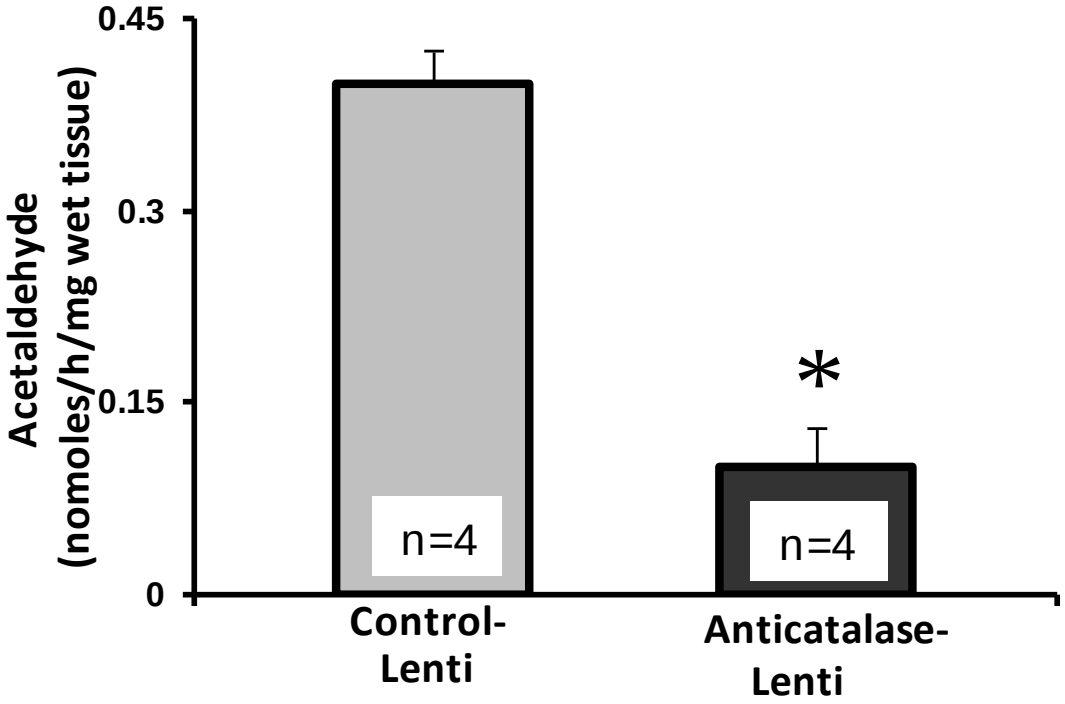


Dopaminergic neurones (VTA)

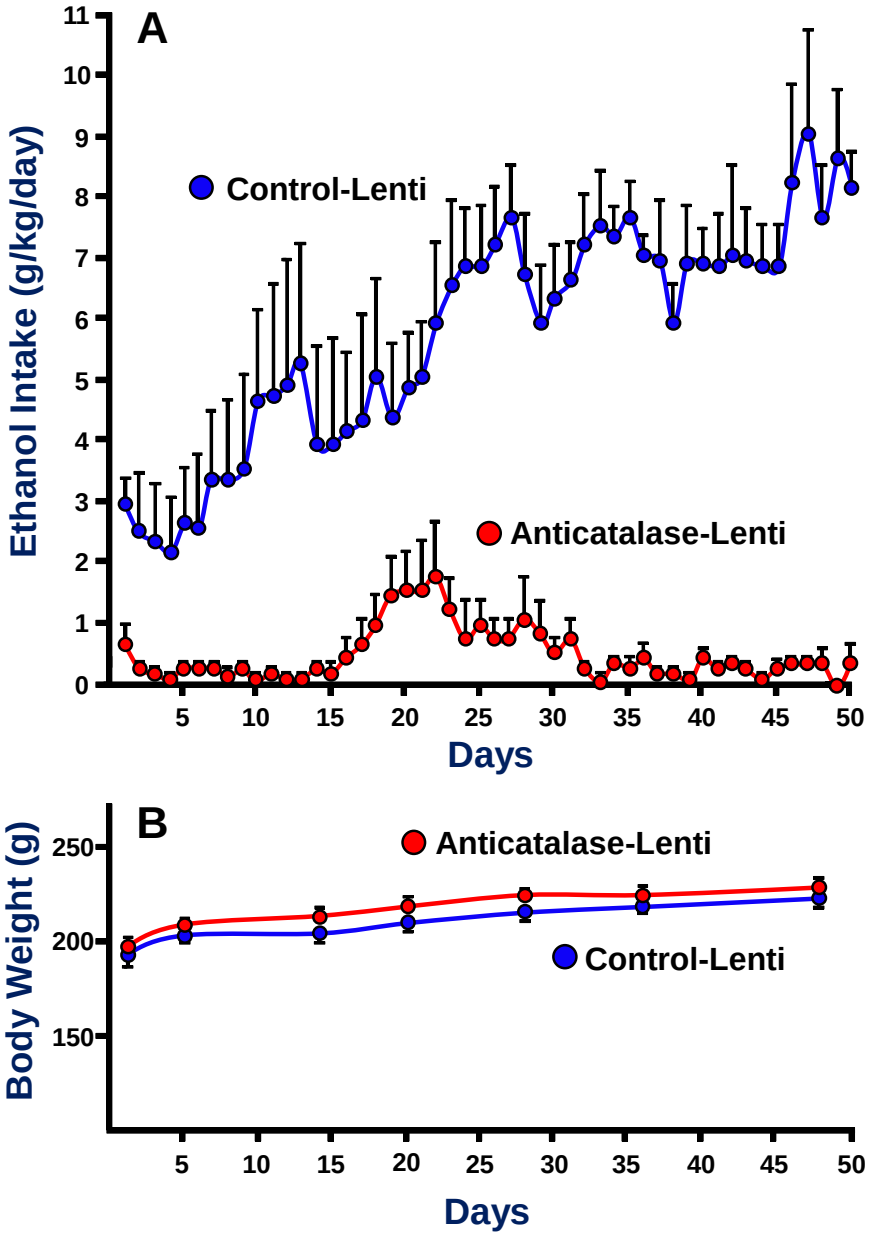


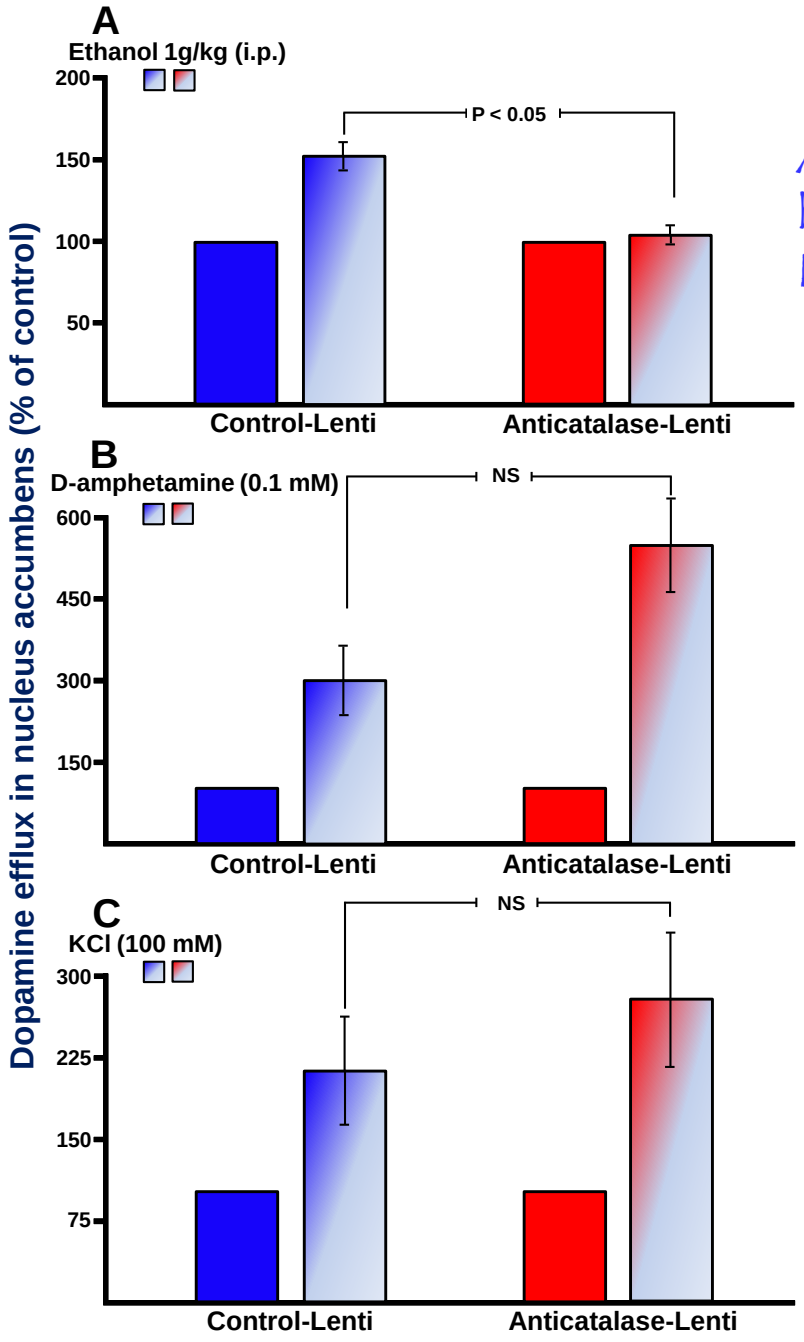
Inhibition of catalase synthesis by microinjection of Lentiviral vector coding for shRNA anticatalase into the VTA

Inhibition of Brain Catalase(VTA) by stereotaxic administration of an anti-catalasa viral vector



Inhibition of alcohol consumption by inhibition of brain acetaldehyde synthesis (lowering catalase synthesis)

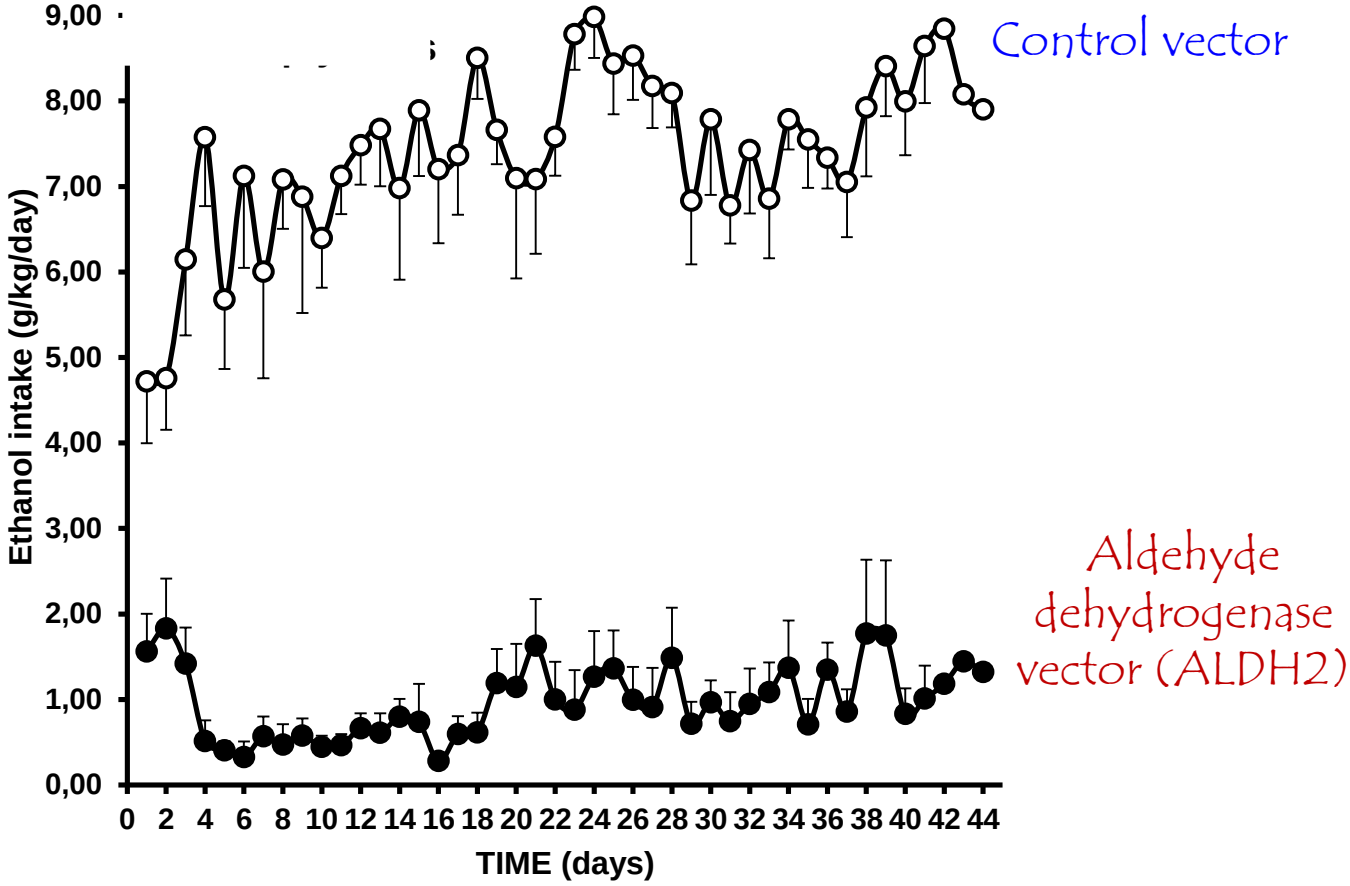




Anticatalase Lentiviral vector specifically blocks the liberation of dopamine in Nucleus Accumbens

Karahanian E et al ePub 2011

Inhibition of alcohol consumption by activation of brain acetaldehyde elimination (by increasing aldehyde de dehydrogenase synthesis)



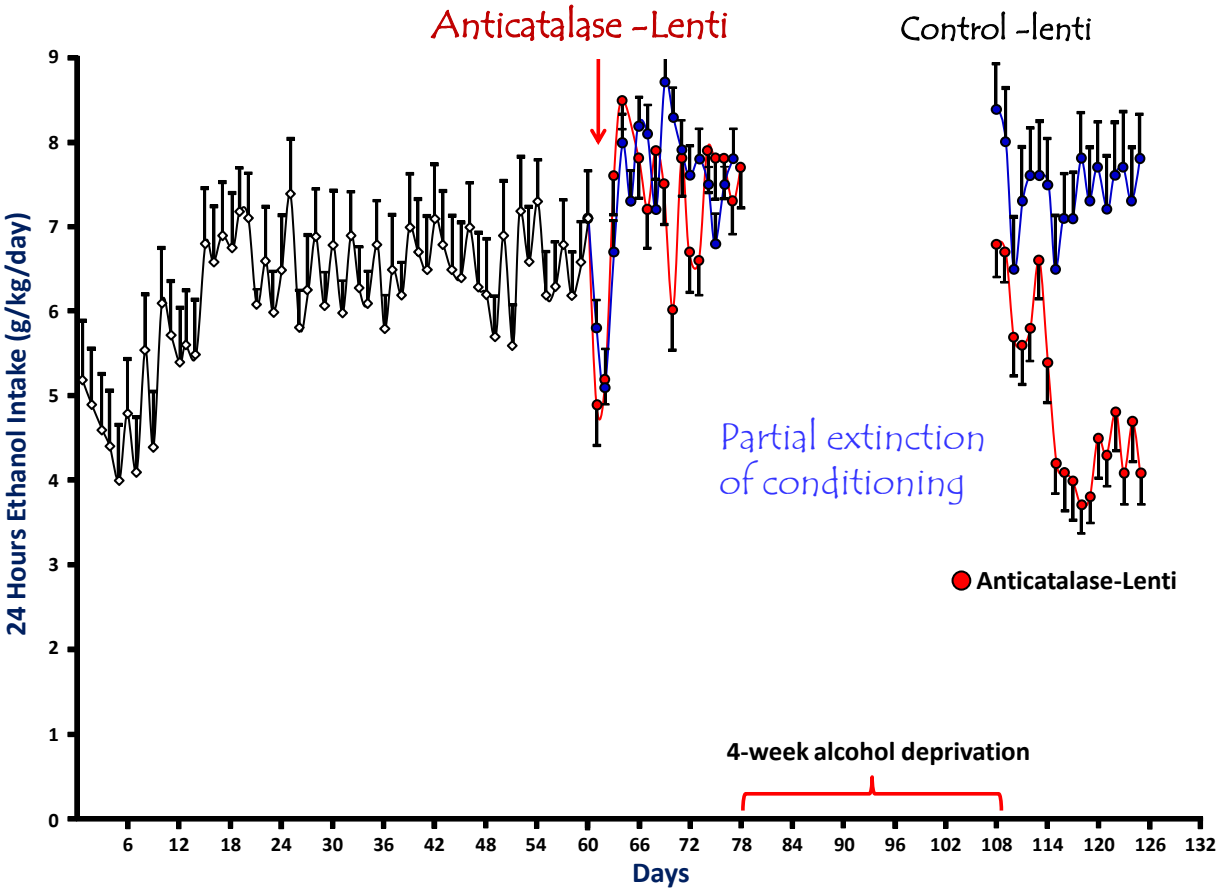
Manuscript in preparation 2012

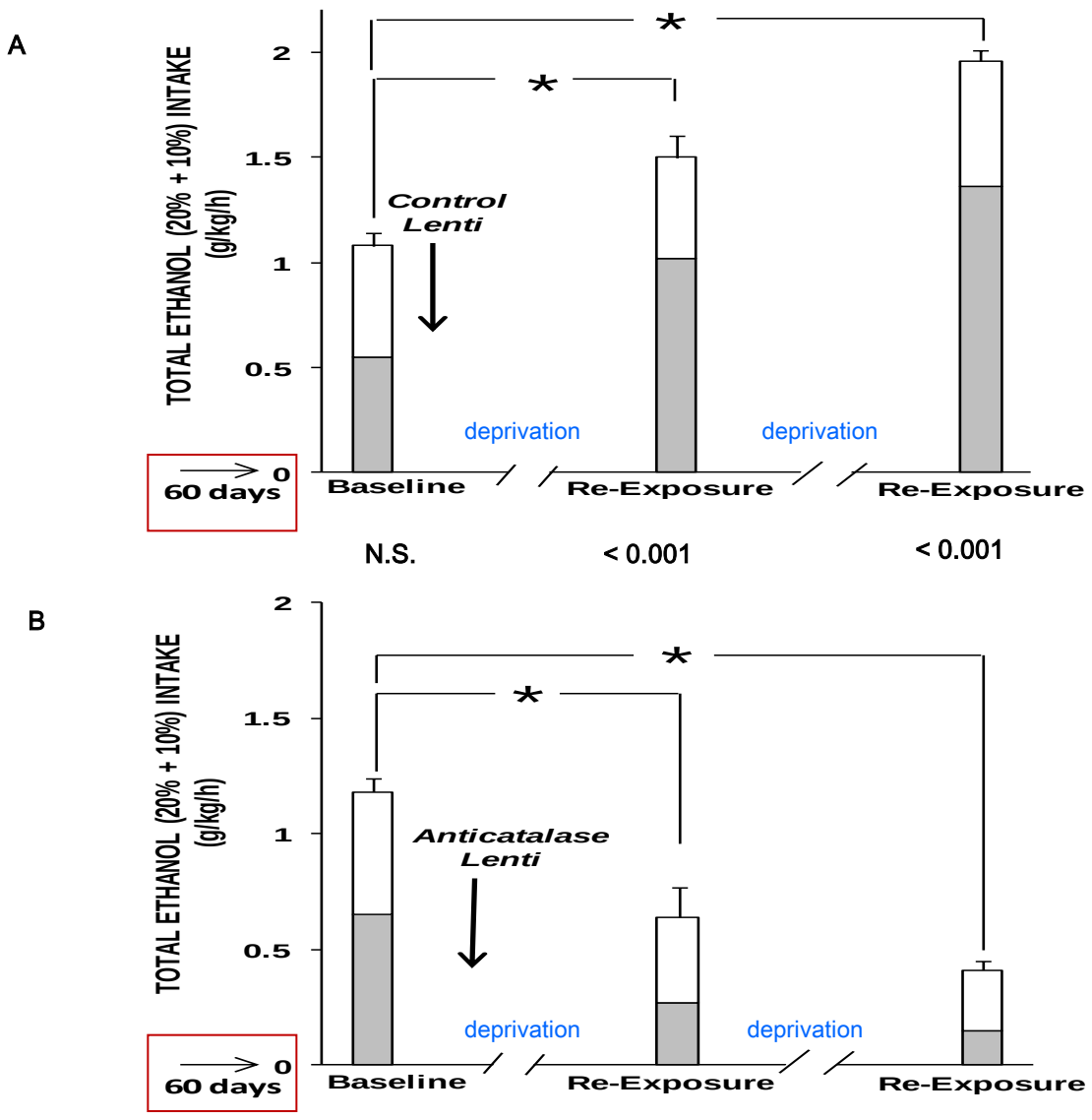
TREATMENT DEALS MOSTLY WITH:

Positive Reinforcement
(increasing aversive effects or
reduce rewarding effects;)

Conditioning (situational)
(memory, stress: "Craving")

Partial extinction of conditioning is needed to observe the inhibitory effect of the anticatalase lentiviral vector on ethanol intake.





Effect of Anticatalase on the Alcohol deprivation effect (ADE) "happy hour"

80% inhibition of "binge drinking" by brain (VTA) anticatalase"

GENE DELIVERY
CONCLUSIONES TO TREAT ALCOHOLISM
FROM PRECLINICAL STUDIES

1. Inhibit acetaldehyde degradation.
   By reduction of liver aldehyde dehydrogenase gene expression (duración 30 days-1 year)

2. Reduce brain catalase synthesis by gene delivery.
   Inhibition of brain acetaldehyde generation.

3. Increase brain aldehyde dehydrogenase synthesis by gene delivery.
   Activation of brain acetaldehyde degradation

4. Reduce conditioning and memory of reward (added to the above).

Agradecimientos

Financiamiento:
NIH, Fondecyt, Instituto Milenio ICDB