Objectives

- to understand
  - mechanism of action of ethanol
  - physiological effects
  - tolerance and hangovers
  - types and mechanism of organ damage

$GABA_A$ receptors - ligand-gated ion channels

Ligand gated ion channel – a receptor whose integral ion channel is opened or closed by a neurotransmitter

- 5 subunits per $GABA_A$ receptor
- most abundant subunits are alphas (6 kinds possible), betas (4) and gammas (3) and delta
- GABA binds at interface between alpha and beta subunits
- central chloride ion channels opens when activated by GABA, chloride ions enter postsynaptic cell
- negative charge on chloride ion hyperpolarizes the membrane (inhibits depolarization)
- alcohol potentiates the effects of GABA
- Only those receptors containing delta subunits rather than a gamma subunit respond to low ethanol levels that most humans would experience
- Alcohol interacts with regions in the receptor that interface with the lipid bilayer

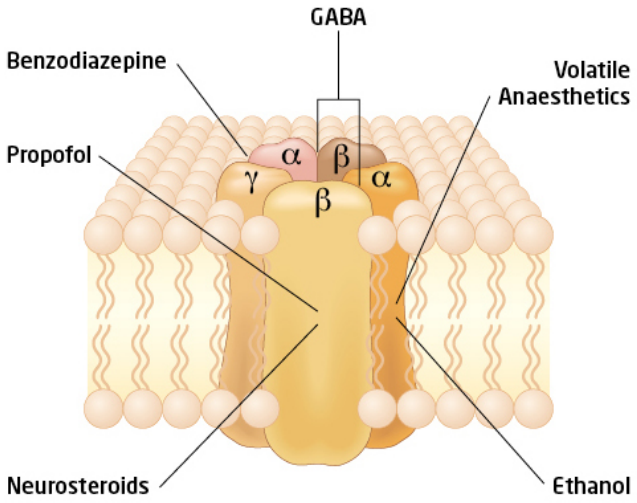


Figure 7.2 Schematic Drawing of the Gamma Aminobutyric Acid ($GABA_A$) Benzodiazepine Receptor Complex

Source: Lovinger, David M. National Institute of Health, National Institute on Alcohol and Alcohol Abuse. *Communication Networks in the Brain*, Figure 4. 2008. Retrieved June 22, 2011, from http://pubs.niaaa.nih.gov/publications/arh313/196-214.htm.

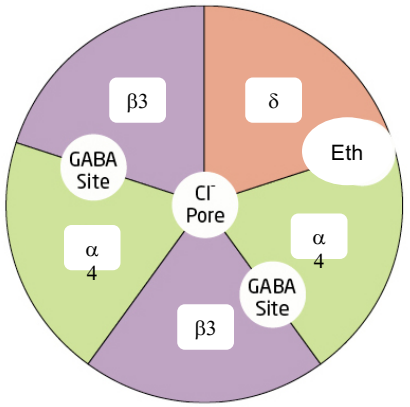
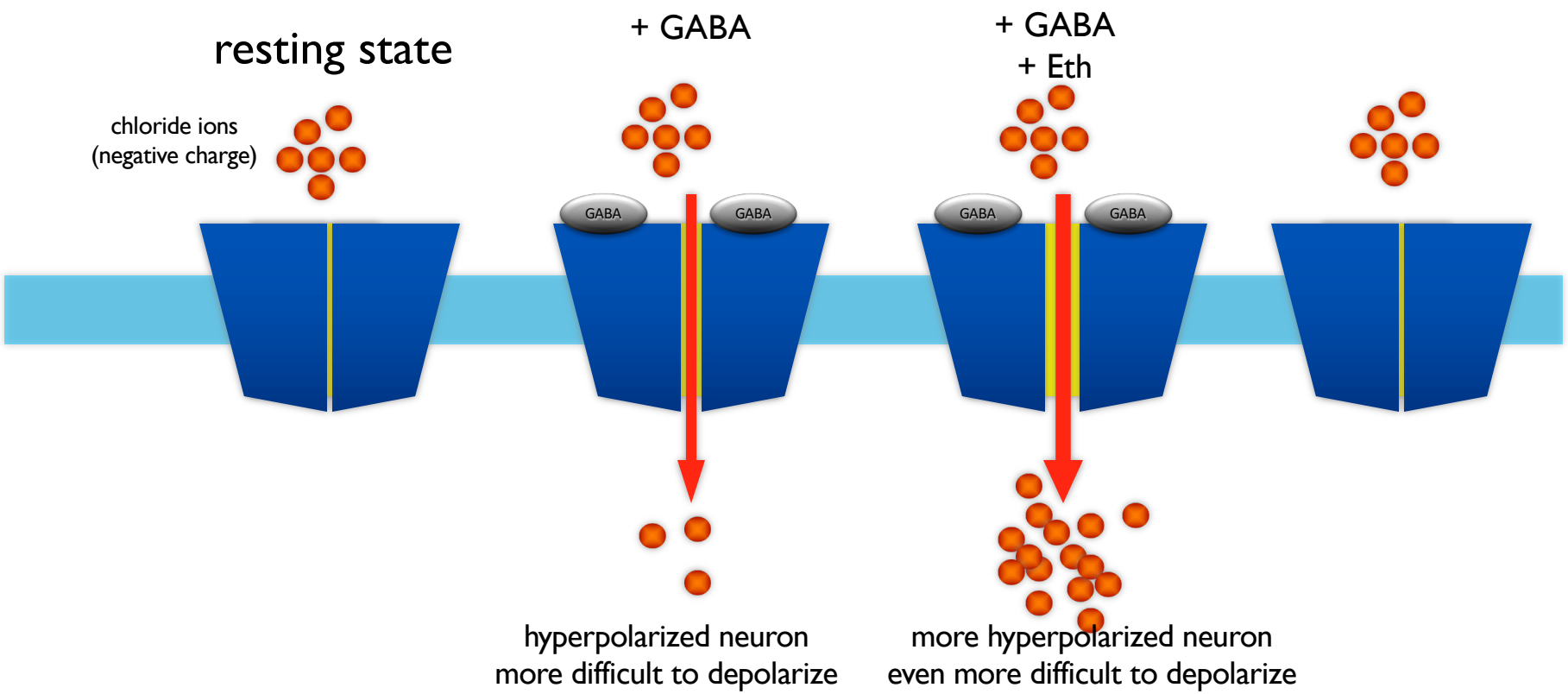


Figure 7.3 Schematic Diagram of a $GABA_A$ Receptor Protein

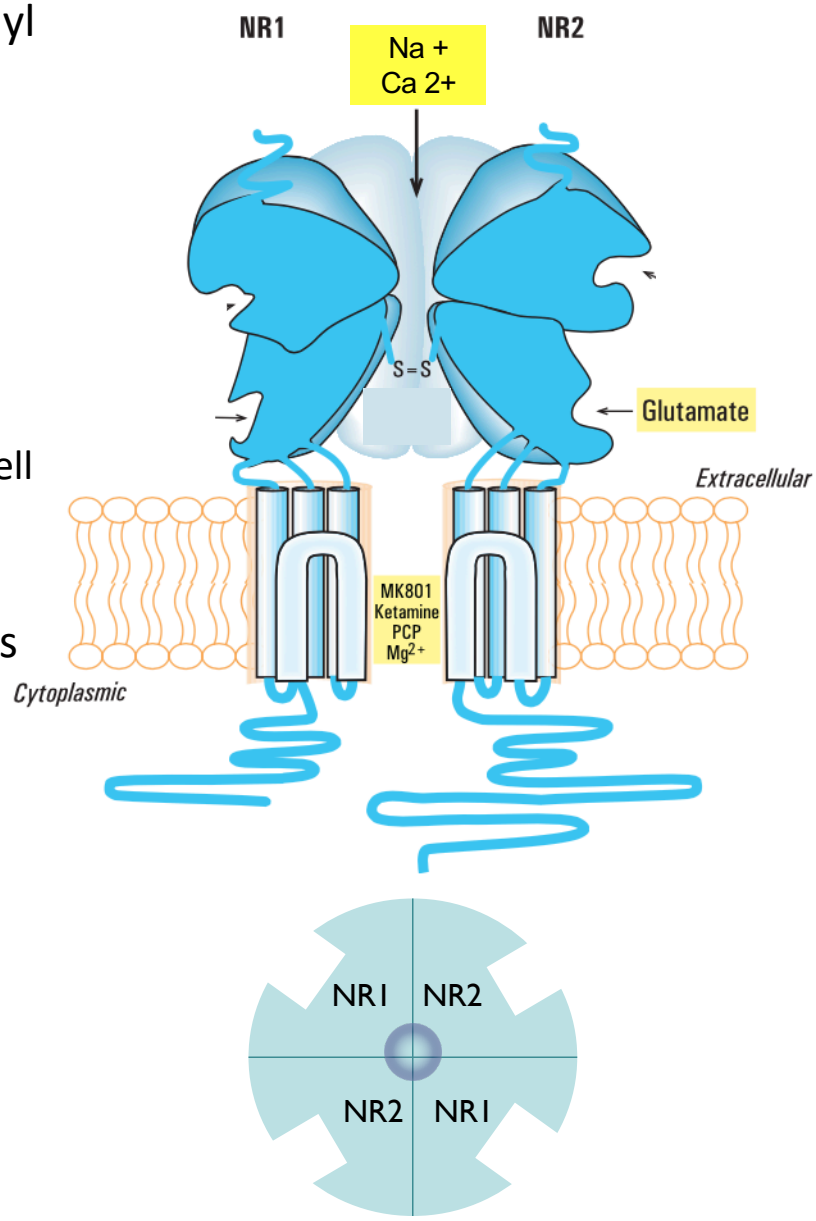
Source: Wikipedia, The Free Encyclopedia. "The $GABA_A$ Receptor." Retrieved November 9, 2011, from http://en.wikipedia.org/wiki/GABAA_receptor.

$GABA_A$ receptors



NMDA receptors (glutamate)

- type of glutamate receptor that is activated by N-methyl D aspartate (NMDA) (synthetic compound used to identify specific glutamate receptors)
- four subunits per receptor - two NR1 (essential) and two of any NR2 (NR2A, B, C or D)
- Glutamate does not bind at subunit interfaces
- central ion channel that is opened upon activation
  - allows for positively charged ions to enter postsynaptic cell (sodium and calcium)
  - this leads to depolarization of the neuron
- ethanol inhibits receptor activity at high concentrations
- Ethanol does not seem to bind to the same site as NMDA – probably in transmembrane domains that interface with the lipid bilayer
- In conditions where receptors are over-activated, get excitoxicity due to excess calcium entering neurons
- This is thought to lead to the neuronal loss seen in alcoholics



Willard and Koochekpour Int J Biol Sci 9:948–959 2013

mechanism of action

- at relatively low doses (but still higher than doses that affect dopamine release), ethanol strongly potentiates effects of GABA at specific $GABA_A$ receptors (containing delta subunits)
  - The GABA-A receptors that respond to low ethanol concentrations (contain δ subunits) may not actually be in the synaptic cleft
- at higher doses ethanol inhibits the effects of glutamate on glutamate receptors while still affecting $GABA_A$ receptors
- ethanol may also inhibit calcium entry through voltage-gated calcium channels at high doses
  - this reduces neurotransmitter release
- net effect - neuronal inhibition – drowsiness, anesthetic-like properties – can shut down basic autonomic functions (breathing) and result in death at high doses

controversy over delta subunits

Ethanol enhances $\alpha_4\beta_3\delta$ and $\alpha_6\beta_3\delta$ γ-aminobutyric acid type A receptors at low concentrations known to affect humans

M. Wallner*, H. J. Hanchar*, and R. W. Olsen†

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The δ Subunit of γ-Aminobutyric Acid Type A Receptors Does Not Confer Sensitivity to Low Concentrations of Ethanol

Cecilia M. Borghese, Signe í Stórustovu, Bjarke Ebert, Murray B. Herd, Delia Belelli, Jeremy J. Lambert, George Marshall, Keith A. Wafford, and R. Adron Harris

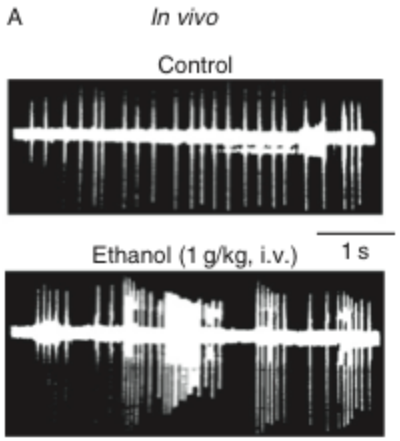
Waggoner Center for Alcohol and Addiction Research, The University of Texas at Austin, Austin, Texas (C.M.B., R.A.H.); Department of Electrophysiology, H. Lundbeck A/S, Valby, Denmark (S.S., B.E.); Neuroscience Institute, Division of Pathology and Neuroscience, Ninewells Hospital and Medical School, Dundee University, Dundee, United Kingdom (M.B.H., D.B., J.J.L.); and Department of Molecular and Cellular Neuroscience, Merck Sharp & Dohme Research Laboratories, The Neuroscience Research Centre, Harlow, United Kingdom (G.M., K.A.W.)

Received July 11, 2005; accepted October 6, 2005

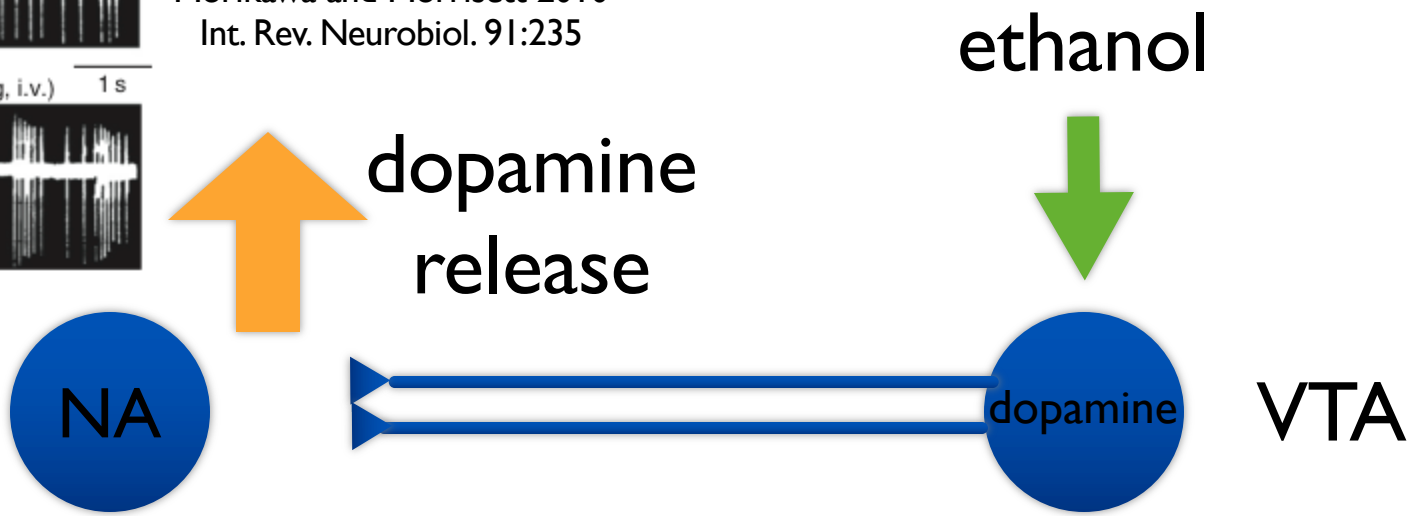
ethanol and the mesolimbic circuit

- administer ethanol directly to the nucleus accumbens
- get little if any increase in dopamine release

ethanol and the mesolimbic circuit



Morikawa and Morrisett 2010
Int. Rev. Neurobiol. 91:235



- administer ethanol to the VTA
- Change from tonic to phasic firing pattern – neurons depolarize with increased frequency
- get increased dopamine release from VTA neurons that synapse with the NA

Paradox

why do VTA dopaminergic neurons fire more often in presence of ethanol when in the rest of the brain ethanol decreases neuronal excitability?

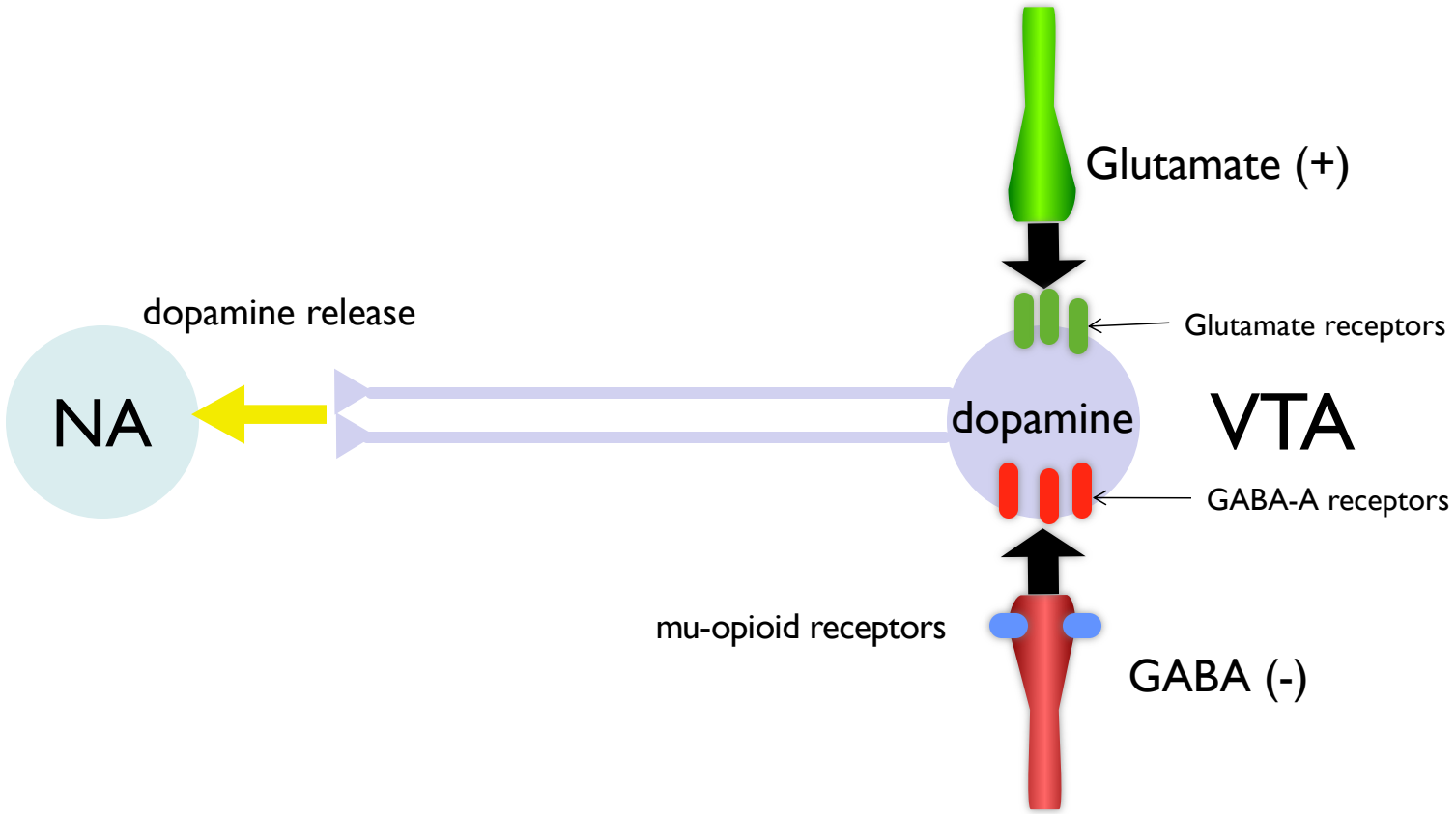
- VTA controlled by glutamate and GABA
- ethanol enhances activity of GABA-A receptors while decreases activity of NMDA receptors

- Shouldn't the net effect be decreased excitability of VTA neurons?

What controls GABA release in VTA?

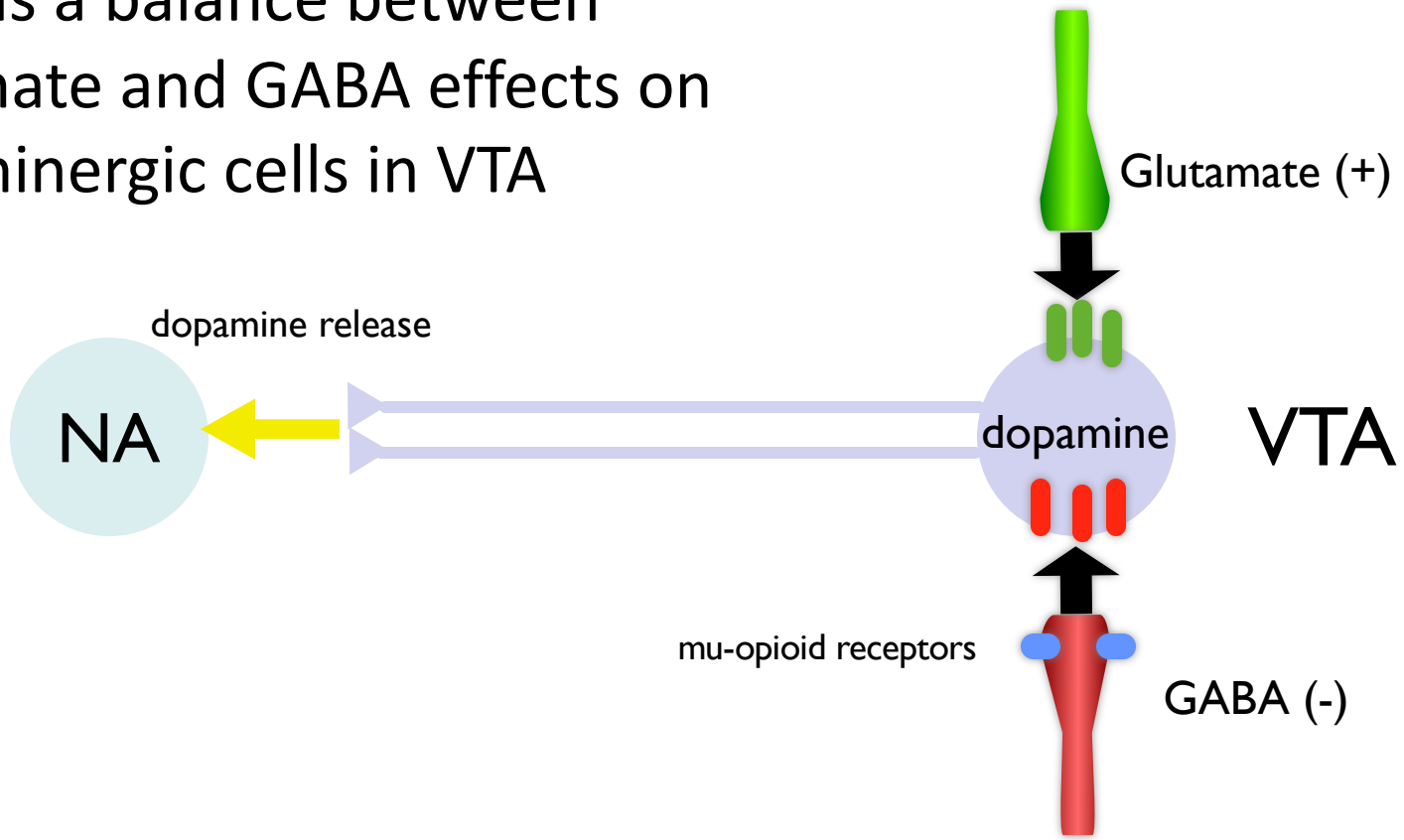
- most interesting - evidence for mu-opioid receptors on GABA-releasing neurons
  - ethanol increases the release of beta-endorphin, a naturally occurring mu opioid agonist from projections that come from the hypothalamus
  - When mu-opioid receptors are activated, they inhibit voltage-gated calcium channel function (calcium is necessary for neurotransmitter release) and increase potassium channel function (allows efflux which hyperpolarizes the membrane)
  - Because of the location of mu-opioid receptors on GABA-releasing neurons in the VTA, less GABA is released

Normal presynaptic control of GABA release



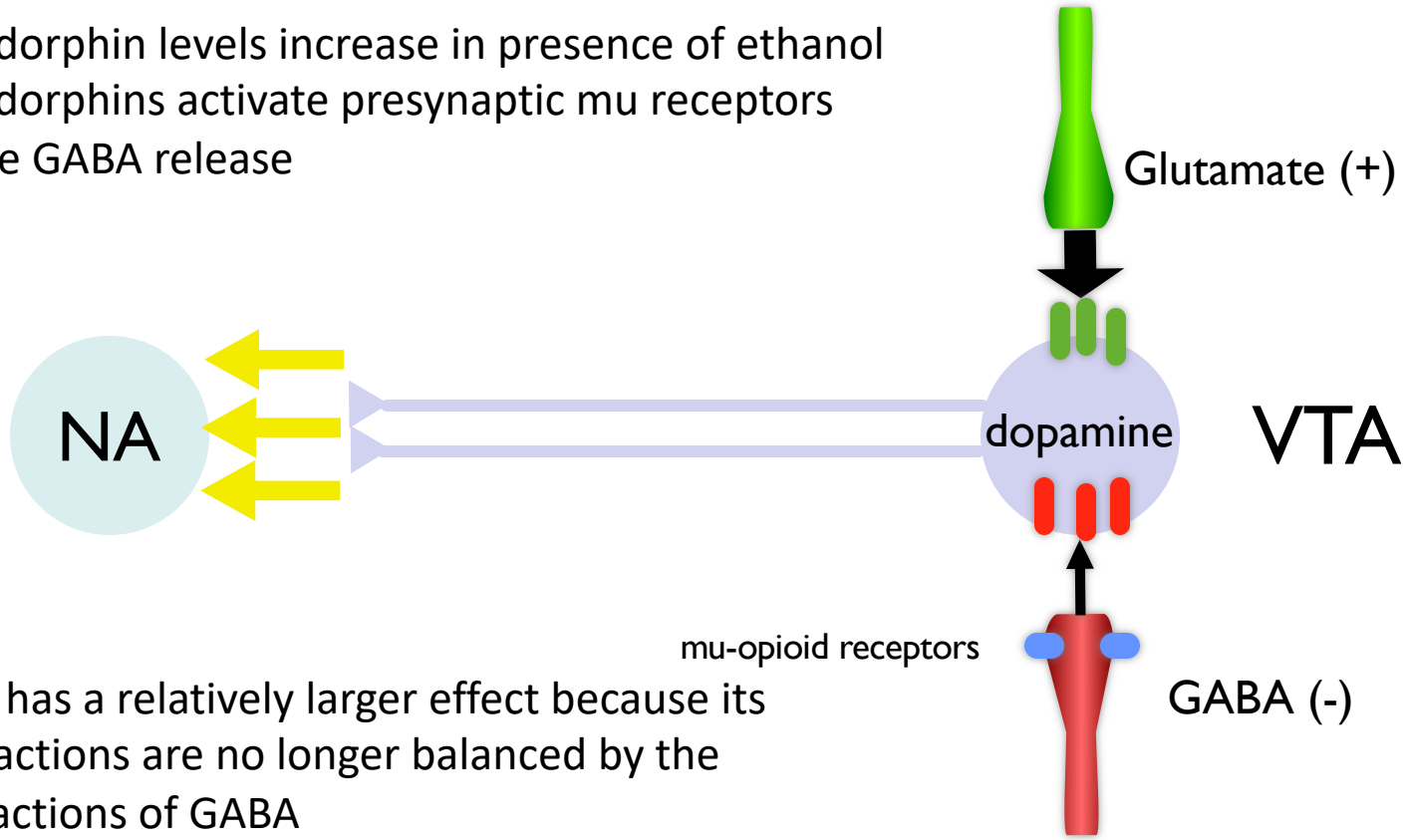
Normal presynaptic control of GABA release

- There is a balance between glutamate and GABA effects on dopaminergic cells in VTA



presynaptic control of GABA release in presence of ethanol

- Beta-endorphin levels increase in presence of ethanol
- Beta-endorphins activate presynaptic mu receptors
- Decrease GABA release



- Glutamate has a relatively larger effect because its excitatory actions are no longer balanced by the inhibitory actions of GABA

physiological effects

- vasodilation through central vasomotor control mechanism in brainstem
  - feeling of warmth, but core temp may decrease
- increased salivary and gastric secretion - promotes hunger in some
- damage to gastric mucosa – bleeding via constant irritation of the stomach lining

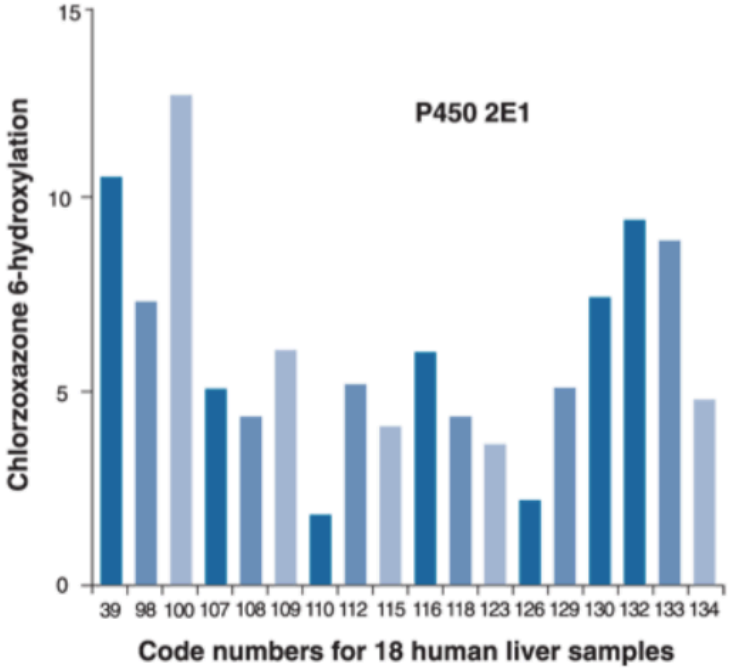
tolerance

- Because alcohol potentiates the action of GABA at $GABA_A$ receptors
  - $GABA_A$ receptor numbers decrease (downregulation)
  - also the types change - different subunits - different alcohol sensitivity?
- Because alcohol inhibits actions of glutamate at NMDA receptors
  - NMDA receptor numbers increase (upregulation)
- Because alcohol inhibits calcium channels
  - calcium channels numbers increase (upregulation)

tolerance

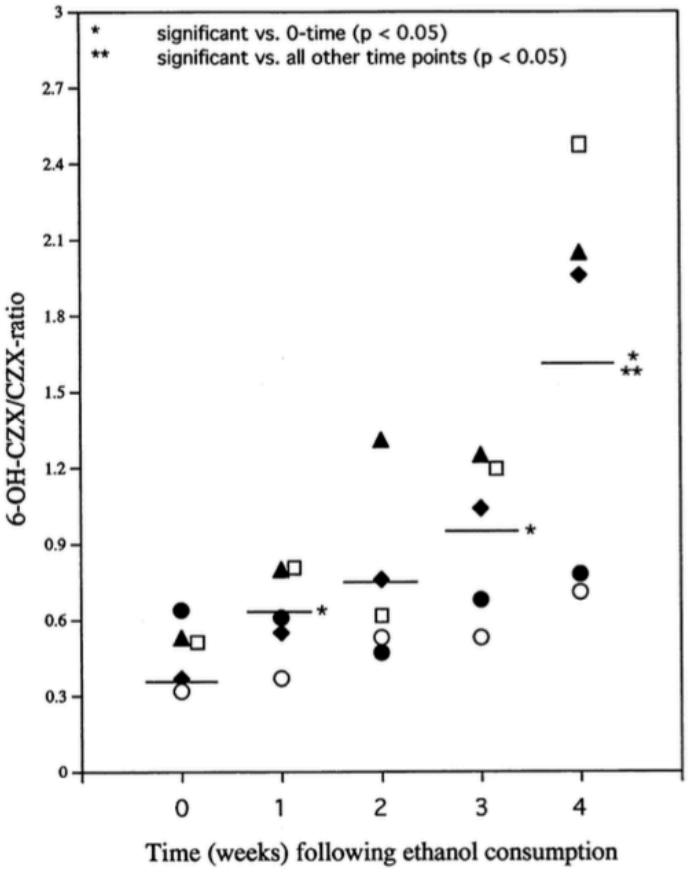
- behavioural tolerance is common
  - Modify behaviours to mask the effects of ethanol
- there is also metabolic tolerance
  - CYP2E1 is upregulated significantly (ends up metabolizing 50- 65% of total ethanol)
  - problem - CYP2E1 metabolizes acetaminophen, industrial solvents and some anesthetics into toxic metabolites - so normal therapeutic dose of tylenol can become extremely toxic due to the production of high levels of a toxic metabolite

Variability of CYP2E1 levels in humans



Guengerich (2006) AAPS J. 8:E101-E111

- Humans in general have highly variable CYP2E1 activity
- Above study showed 8X variability



- In this study, healthy volunteers given 40 g of ethanol a day for 5 weeks
- Other experiments have shown as CYP2E1 levels increase, the levels of reactive oxygen species also increase
  - CYP2E1 knockouts in mice show less ethanol-induced liver damage
  - Mice engineered to have higher CYP2E1 levels show more ethanol-induced liver damage

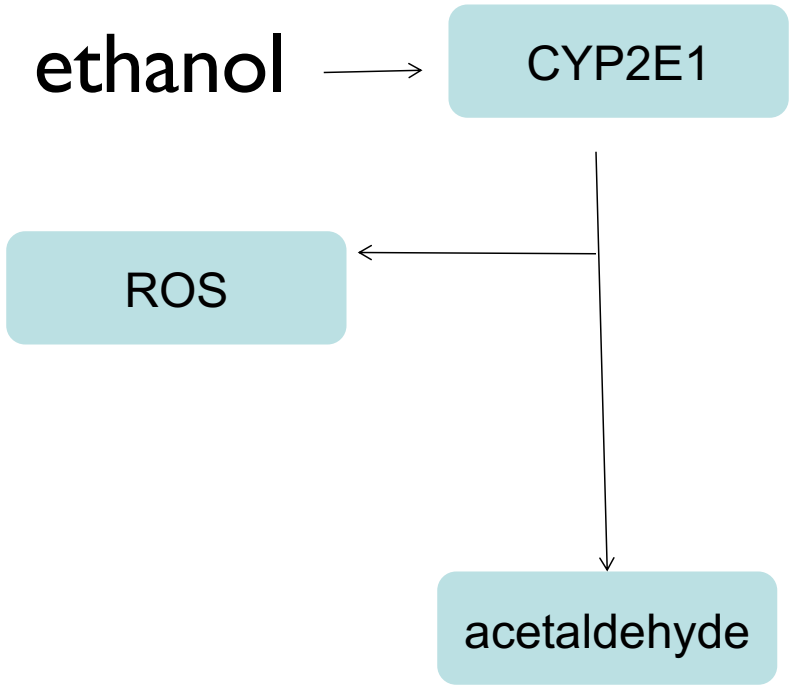
reactive oxygen species

- up-regulated CYP2E1 is one of the major producers of reactive oxygen species (ROS) in liver and other organs
- ROS can interact with and damage:
  - DNA
  - Lipids
  - Proteins

- CYP2E1 uses molecular oxygen in the normal process of metabolizing ethanol into acetaldehyde
- CYP2E1 can produce ROS as a byproduct if the reaction does not go to completion and the oxygen does not get incorporated into the ethanol molecule

CYP2E1 can produce reactive chemical species

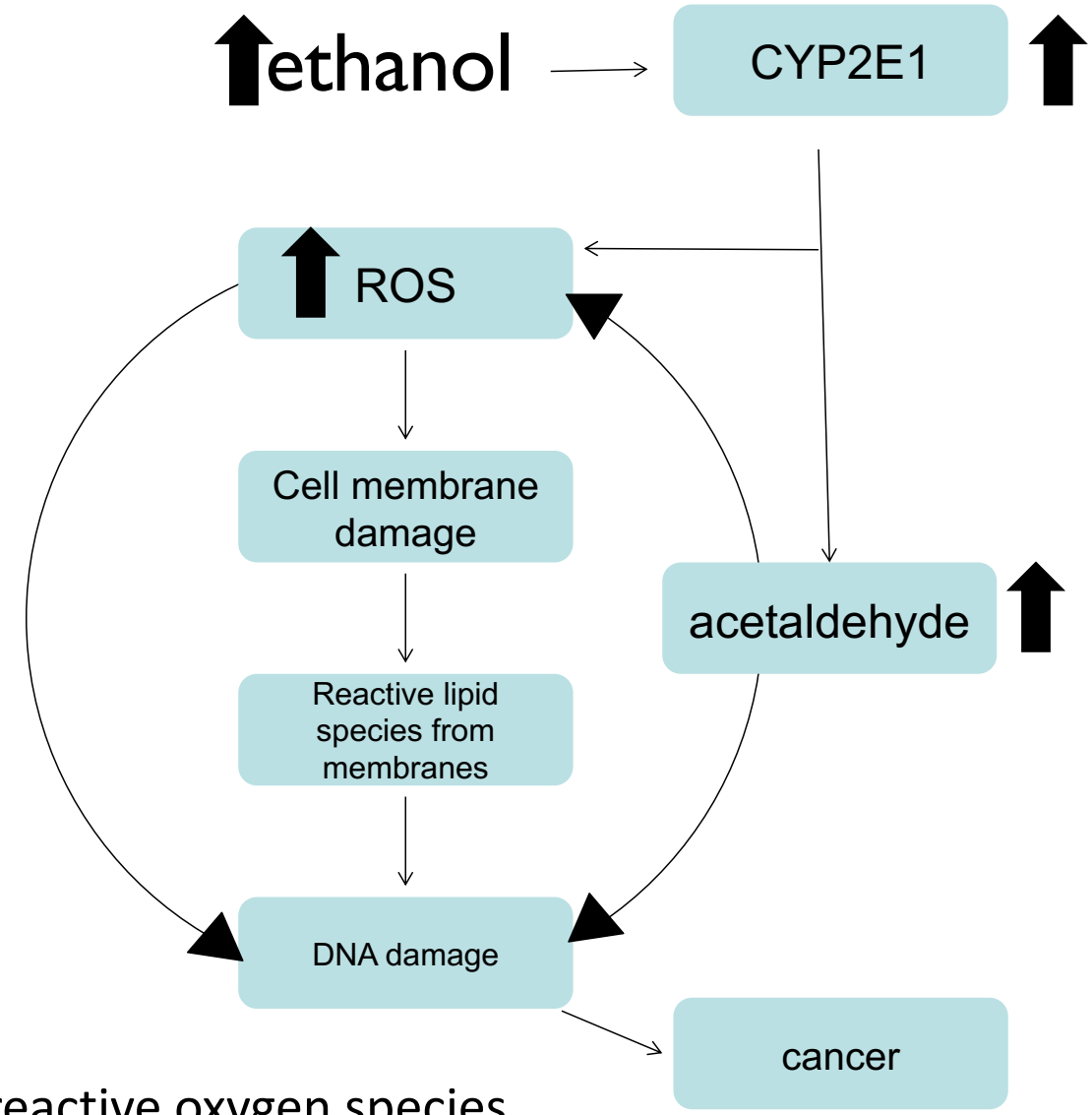
- A small amount of ethanol metabolized by normal levels of CYP2E1 enzyme produces some reactive chemicals
- But the body has a number of mechanisms that detoxify these chemicals



ROS – reactive oxygen species

CYP2E1 can produce reactive chemical species

- After chronic ethanol use, higher CYP2E1 activity results in high levels of ROS that body cannot detoxify
- This leads to membrane damage and cell death
- DNA damage can lead to cell death and/or cancer



ROS – reactive oxygen species

hangovers

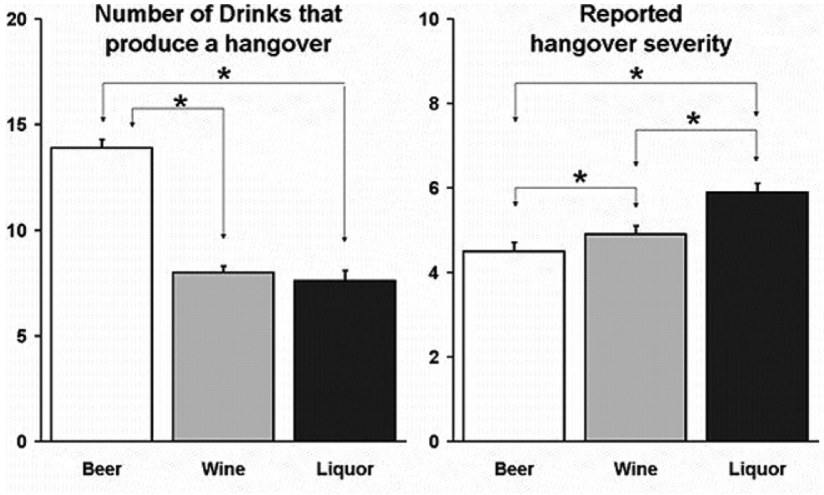
- Physical and psychological symptoms
  - Physical - Headache (66%), diarrhea (36%), fatigue (20%), tremulousness (20%), nausea (9%)
  - Psychological - decreased cognition, impaired visual-spatial skills
- are they withdrawal symptoms from short-term dependence?
- Symptoms peak when BAC = 0%
- Cognition may be impaired even though undetectable levels of ethanol in plasma
- Studies show impairment in functioning of pilots, drivers, skiers

Hangovers – causes?

- dehydration probably not the main cause as rehydration has only a small effect on decreasing symptoms
- some evidence that hangover increases cytokine production via a thromboxane B2 pathway
- cytokines important in immune cell signaling and function
- Many hangover symptoms resemble viral infections which also increase cytokine levels
  - direct injection of cytokines causes many hangover symptoms
  - Prostaglandin synthesis inhibitor, tolfenamic acid, decreases some symptoms by ultimately preventing cytokine production
- Some evidence of mitochondrial dysfunction, especially in cerebellum where integration of sensory and motor pathways occur
  - This can lead to neuronal dysfunction or death

Hangover severity and congeners

- Congener – toxic byproducts of alcohol production and/or storage
  - Congeners include: Acetone, methanol, acetaldehyde, tannins, furfural, fusel oil
- Dark drinks tend to contain more
- Bourbon has 37X the amount as vodka and data suggests more severe hangovers as a result
- Associated with increased severity of hangover symptoms



Verster, 2008 Alcohol Alcohol. 43(2):124

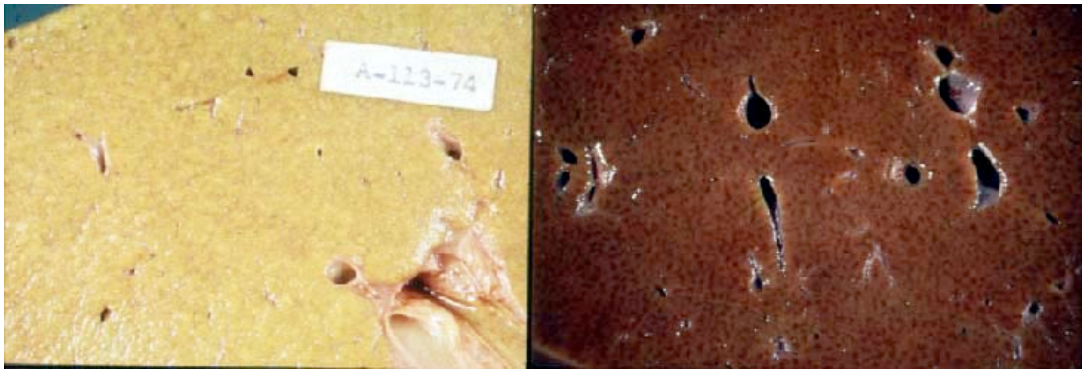
empty calories – ethanol and energy use

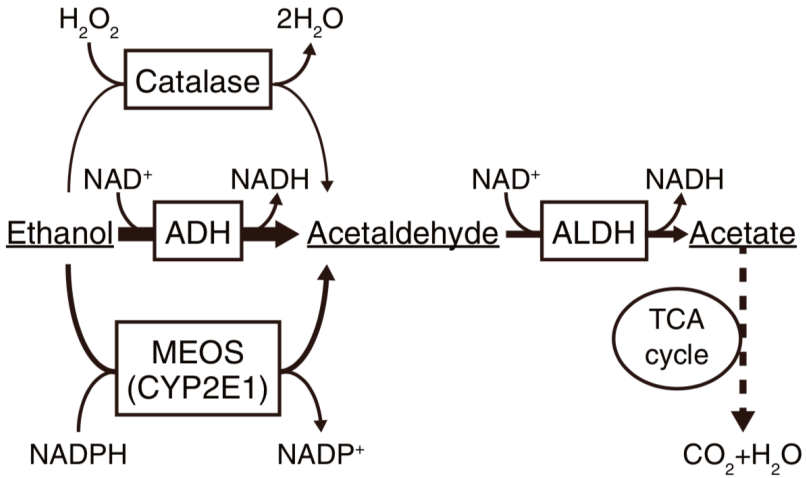
- Alcohol (7 calories/g) is more calorie dense than carbs or protein (both 4 cal/g) and slightly less dense than fat (9 cal/g)
  - Ave. male Canadian drinker – 13.3% (333 cal) of total daily calories come from alcohol
  - Ave female Canadian drinker – 8.2% (159 cal)
- In the brains of heavy drinkers, neuronal energy source can switch from glucose to acetate during intoxication
- When both are exposed to the same amount of ethanol, heavy drinkers metabolize less glucose than non-drinkers in brain tissue when intoxicated

1 beer = 140 cal

1 Cider, Cooler = 220 cal

fatty liver/cirrhosis





- ethanol metabolism by alcohol dehydrogenase converts NAD+ into NADH
- A high NADH:NAD+ ratio is a signal to synthesize fatty acids and stop fatty acid oxidation (usually used for energy) because it occurs when there is an abundant energy source
- The synthesized fat is stored as droplets in hepatocytes and can get to the point where it causes the cell to lyse and cause inflammation
- A fatty liver can be reversible as long as it hasn't progressed to a point where significant cell death is occurring

Cirrhosis

- Cirrhosis usually follows fatty liver if drinking continues (non-reversible)
- Cirrhosis occurs due to a chronic inflammatory state and cell death
- At this stage there is a production of the cytokine, transforming growth factor beta (TGF-β) in liver cells
  - When TGF-β binds to its receptors on the surface of liver cells it can stimulate intracellular pathways that affect gene transcription
  - This lead to the stimulation of collagen synthesis
- Eventually, functional liver cells are replaced by non-functioning connective tissue, mostly collagen
- As the functionality of the liver changes, it loses it ability to detoxify the blood

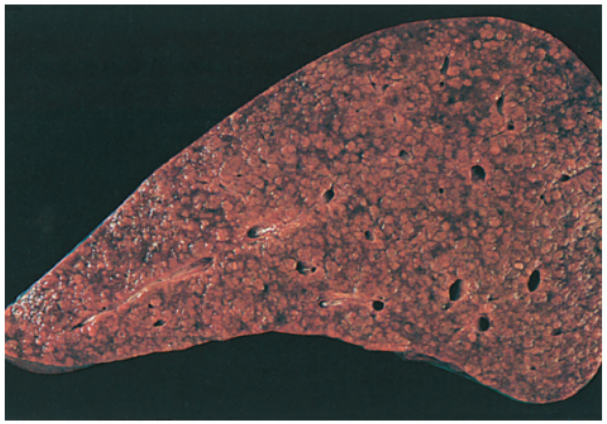


Figure 9.6 **(a)** Normal Liver **(b)** Cirrhotic Liver

immune system and liver inflammation

- ROS and acetaldehyde can modify lipids and proteins
  - body sees them as “foreign”
  - develops antibodies to them - explains involvement of immune system in alcoholic liver disease and contributes to the liver being in a chronically inflamed state
- Ethanol exposure also causes liver cells to release chemical signals (cytokines) that attract immune cells by binding to specific cytokine receptors on these cells
- Immune cells infiltrate the liver and release reactive oxygen and reactive nitrogen species as well as enzymes that destroy liver cells – these things are normally used to kill invading pathogens