

## CONSORTIUM FOR THE STUDY OF THE AMERICAN COLLEGE STUDENT

*A National Think Tank for the Health and Productivity of Our College Students*



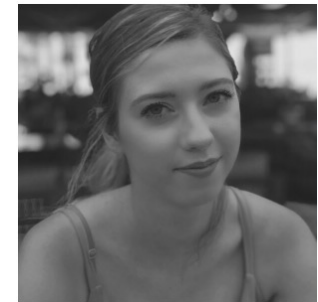
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Linda Spear, Ph.D.  
1950-2020

# Adolescence: Building a Better Brain



## Many Influences on Adolescent Brain Development:

Drug Effects

Nutrition

Sleep

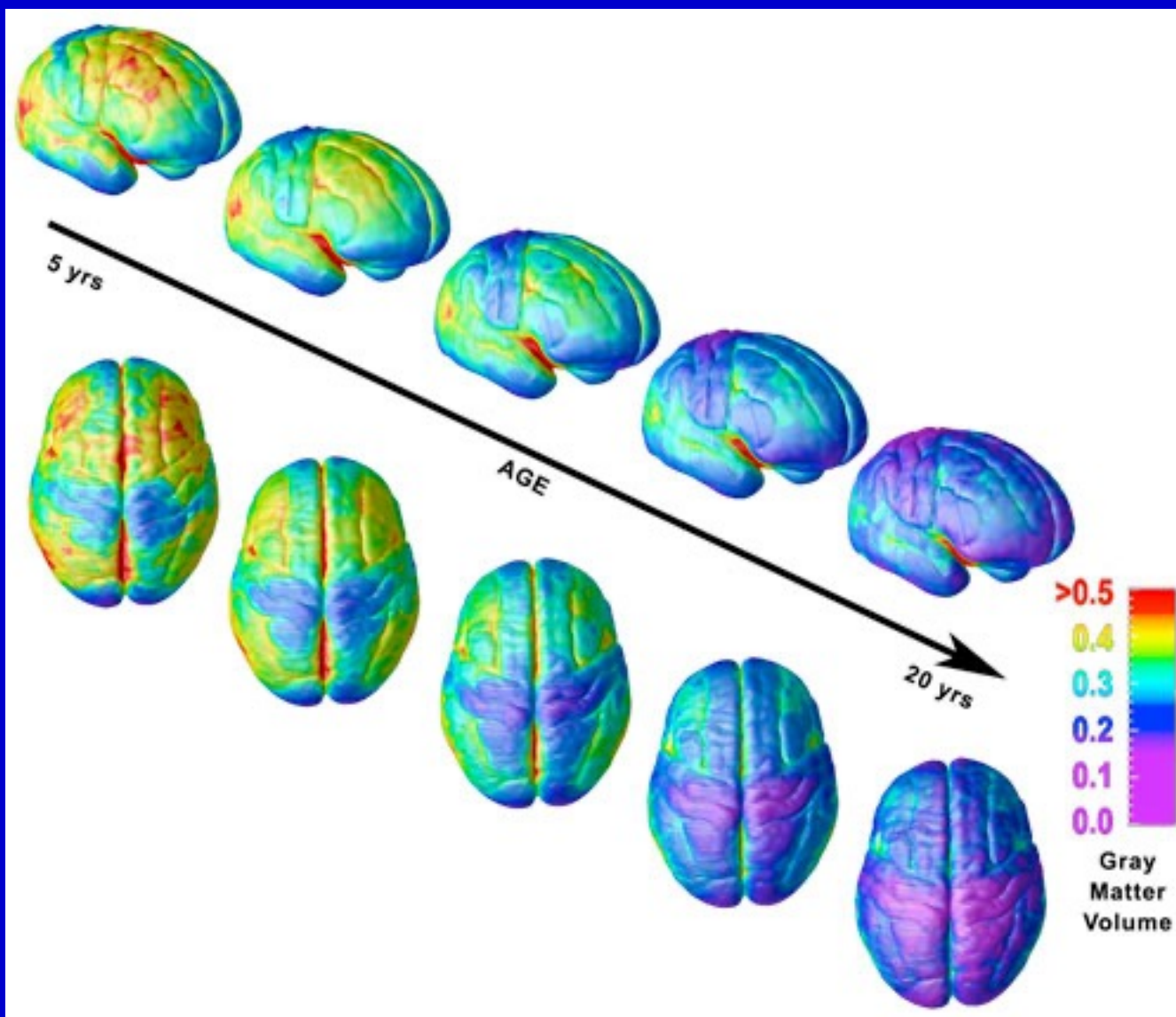
Exercise – physical and cognitive

Stress/trauma

Social Experiences and Learning

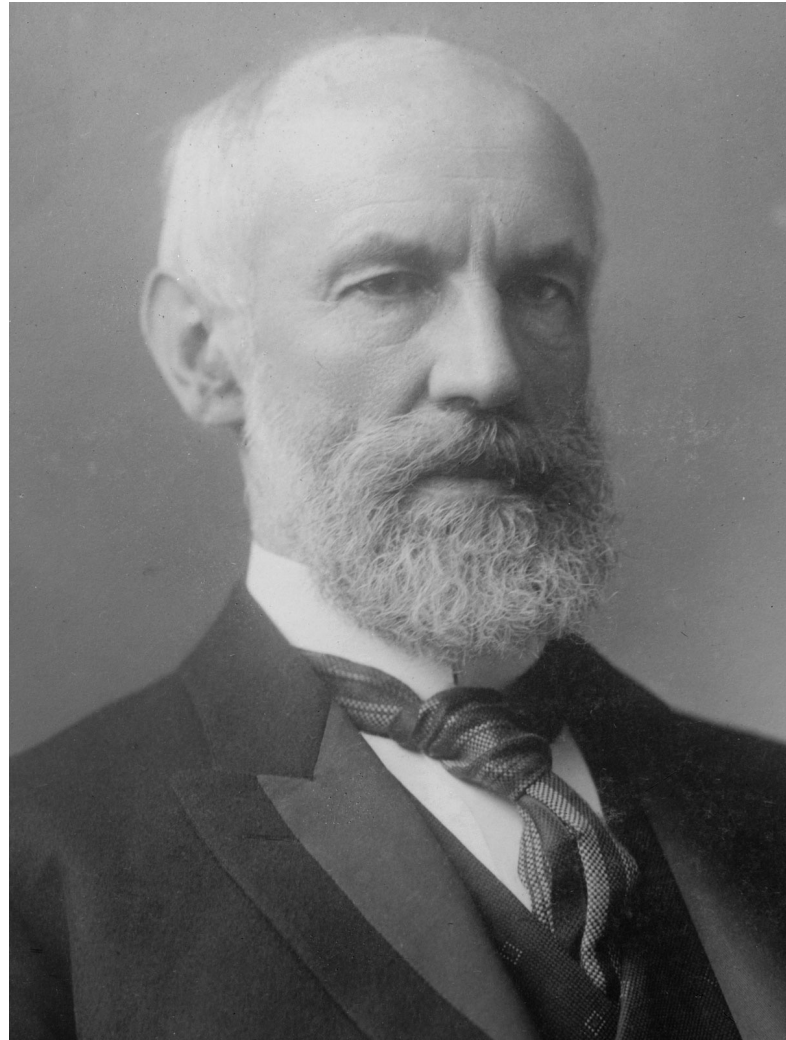
# Adolescent Brain Development

## Neocortical and Subcortical



- Memory
- Problem Solving
- Cognitive Flexibility
- Affect/ Cognition
- Planning

# G. Stanley Hall



“Storm and stress” - 1904

# What's Distinctive About Adolescence?

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Adolescent brain is 'built to learn'

Learning is critical for success during this period.

Learning during this period is critical for success in adulthood

Memories and skills formed are long-lasting

Brain development: Cognition, Affect, Reward, EF

Differential drug sensitivity

# Double-edged sword

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- Enhanced opportunity for learning
- Enhanced vulnerability to disruptive effects of some drugs:
  - Alcohol
  - Cannabinoids
  - Nicotine



# Core Neurobehavioral Effects of Ethanol

Learning: Building block of higher order cognitive function

Sedation: Consciousness, motor function, drinking regulation.

Anxiolysis: Affect, disinhibition, risk-taking

All are differentially regulated by ethanol in adolescence

# Acute EtOH and Memory Continuum of Encoding Deficits

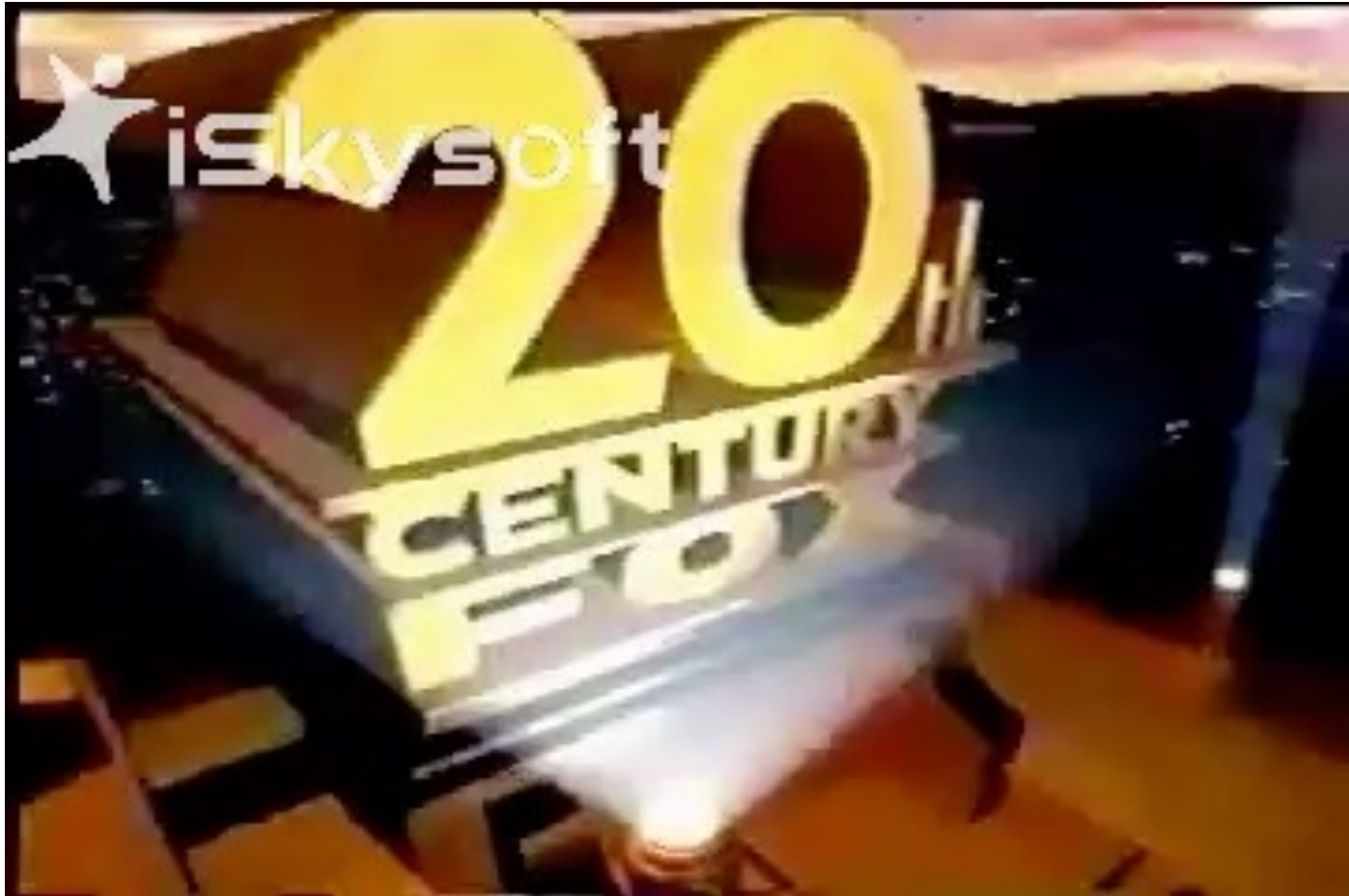
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Mild explicit  
memory  
impairments

Total explicit  
memory  
impairments  
(i.e., blackouts)

# Blackouts

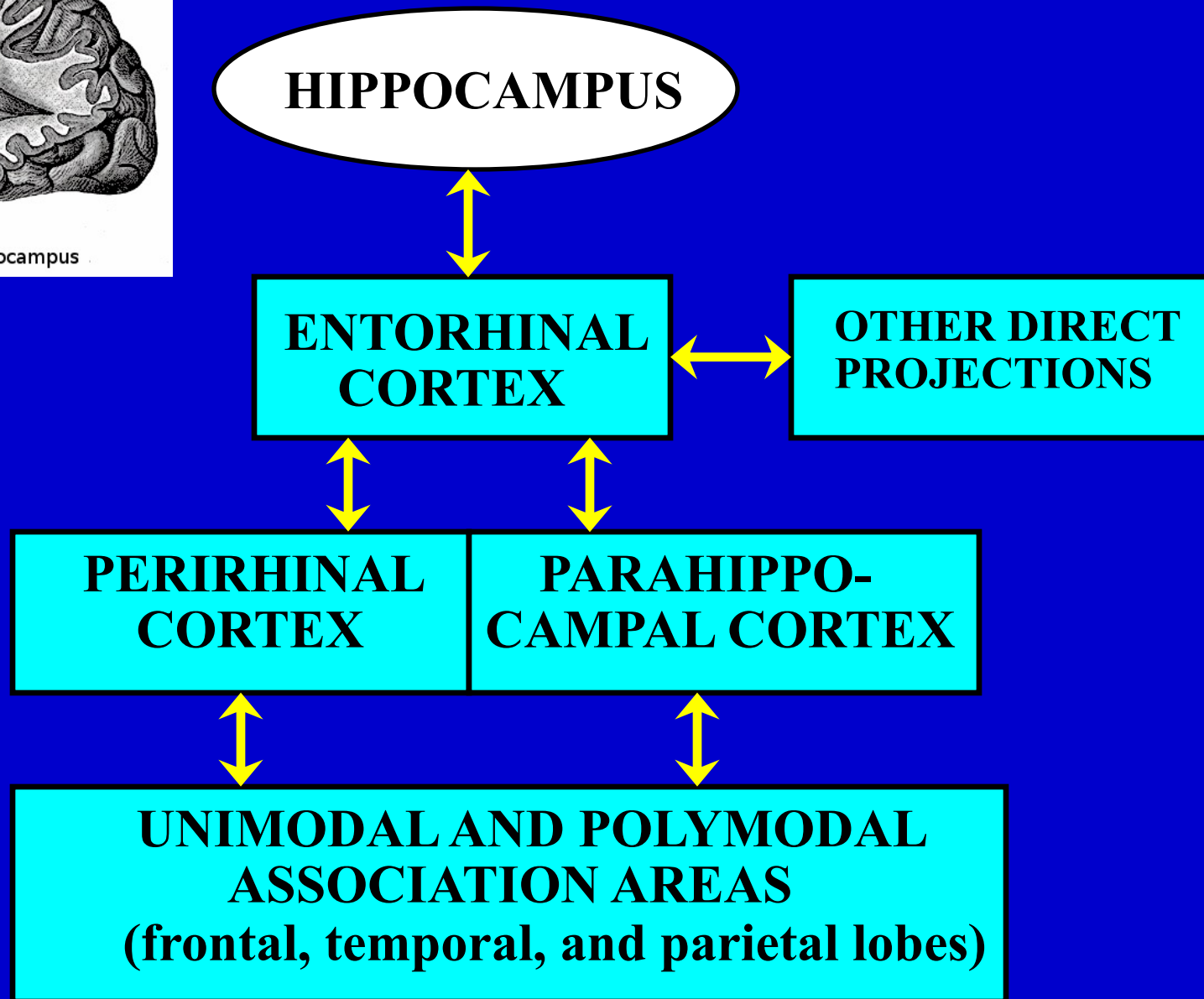
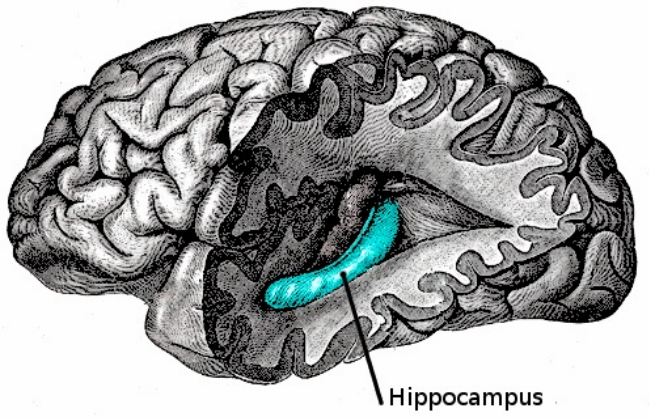


# Events that Occurred During Blackouts

Event	Females <sup>a</sup>	Males <sup>b</sup>	Total <sup>c</sup>	X <sup>2</sup>
	%	%	%	
Insulted someone	30.4	36.0	33.0	X <sup>2</sup> (1) = 1.26,
Spent money not intended to be spent	20.4	35.4	27.3	X <sup>2</sup> (1) = 9.93**
Engaged in some form of sexual activity	24.6	25.0	24.8	X <sup>2</sup> (1) = 0.07
Involved in an argument or fight	15.2	17.7	16.3	X <sup>2</sup> (1) = 0.40
Vandalized property	4.7	29.6	16.1	X <sup>2</sup> (1) = 39.48***
Had unprotected intercourse	4.2	8.5	6.2	X <sup>2</sup> (1) = 2.87
Had unwanted intercourse	5.8	4.3	5.1	X <sup>2</sup> (1) = 0.41
Drove a car	1.6	3.7	2.5	X <sup>2</sup> (1) = 1.56
Was arrested	0.0	0.0	0.0	---

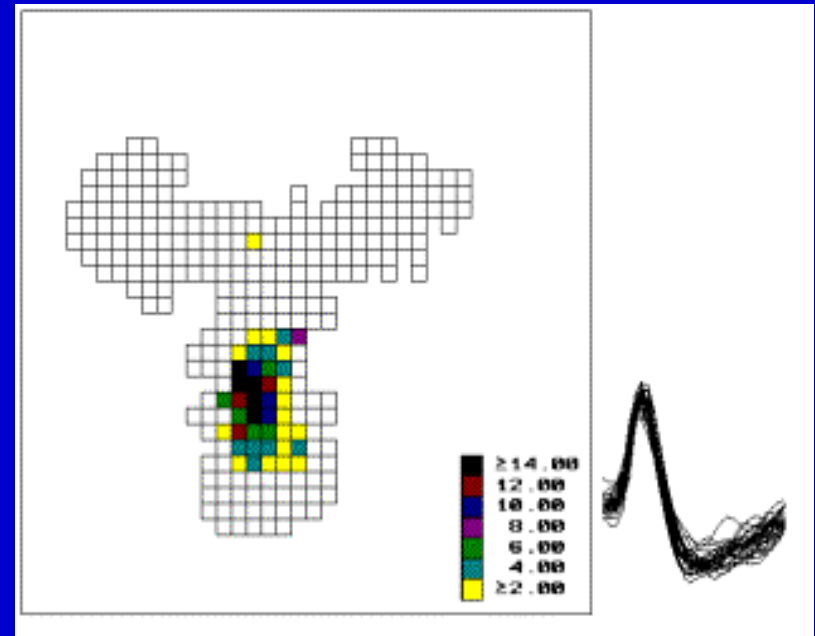
Note. Based upon those who reported having at least one blackout at some point in their lives. In all, 55.5% of students who had at least one blackout later found that one or more of the above events occurred during a blackout.

a, n = 191. b, n = 164. c, n = 355. \*\*p < 0.01. \*\*\*p < 0.001.



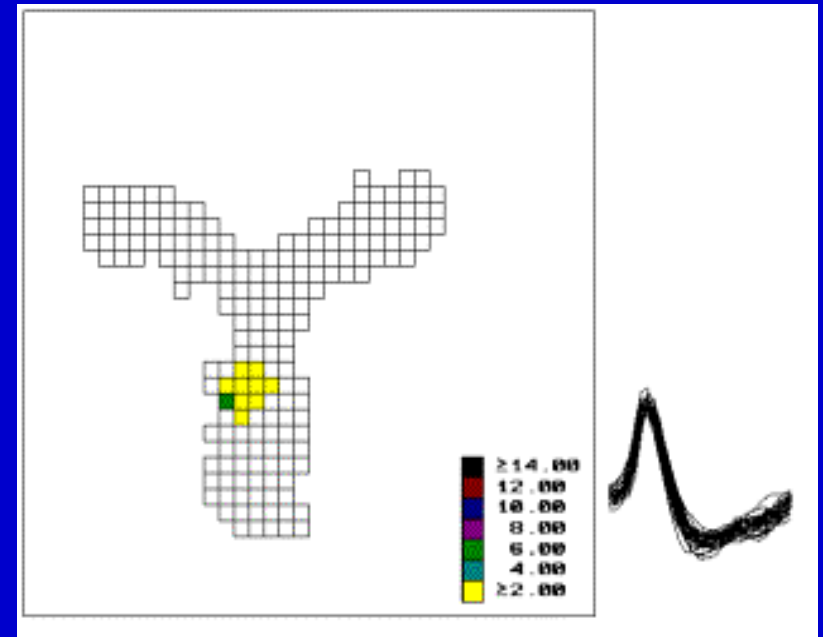
# Hippocampal cell firing before alcohol

## Firing map before alcohol

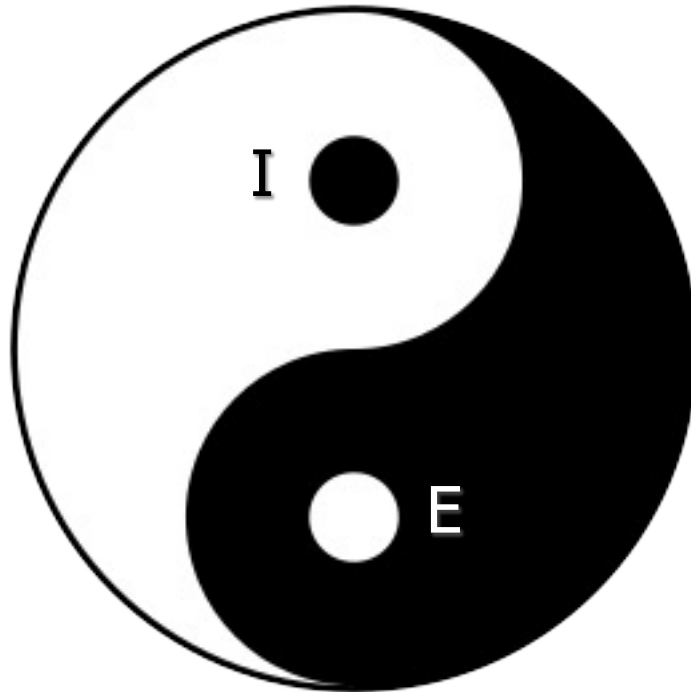


# Cell firing under 1.0 g/kg ethanol

Firing map 30  
min after  
alcohol

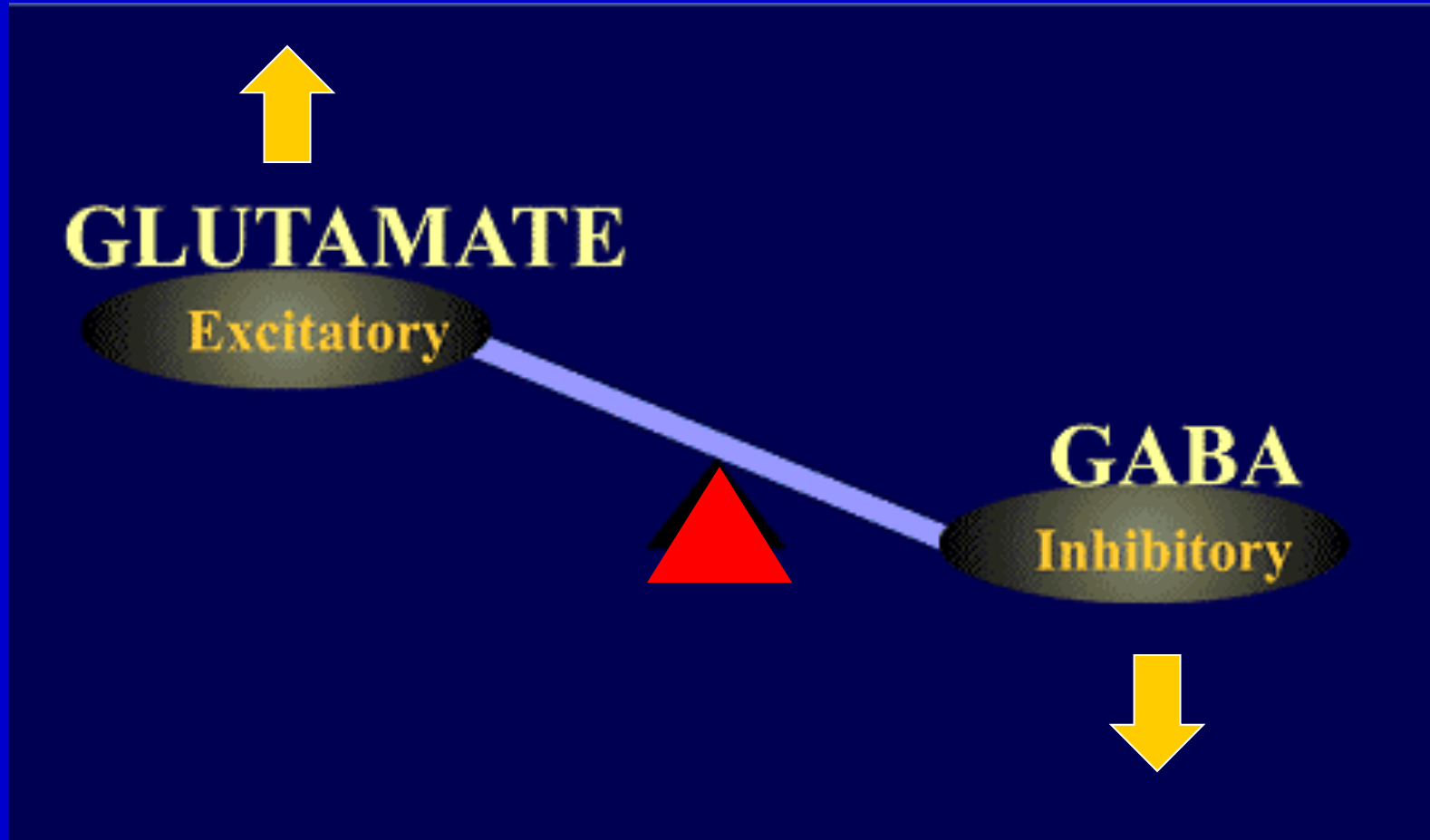


Hippocampal circuits maintain a delicate balance of excitation and inhibition that is critical for cognitive and affective functions



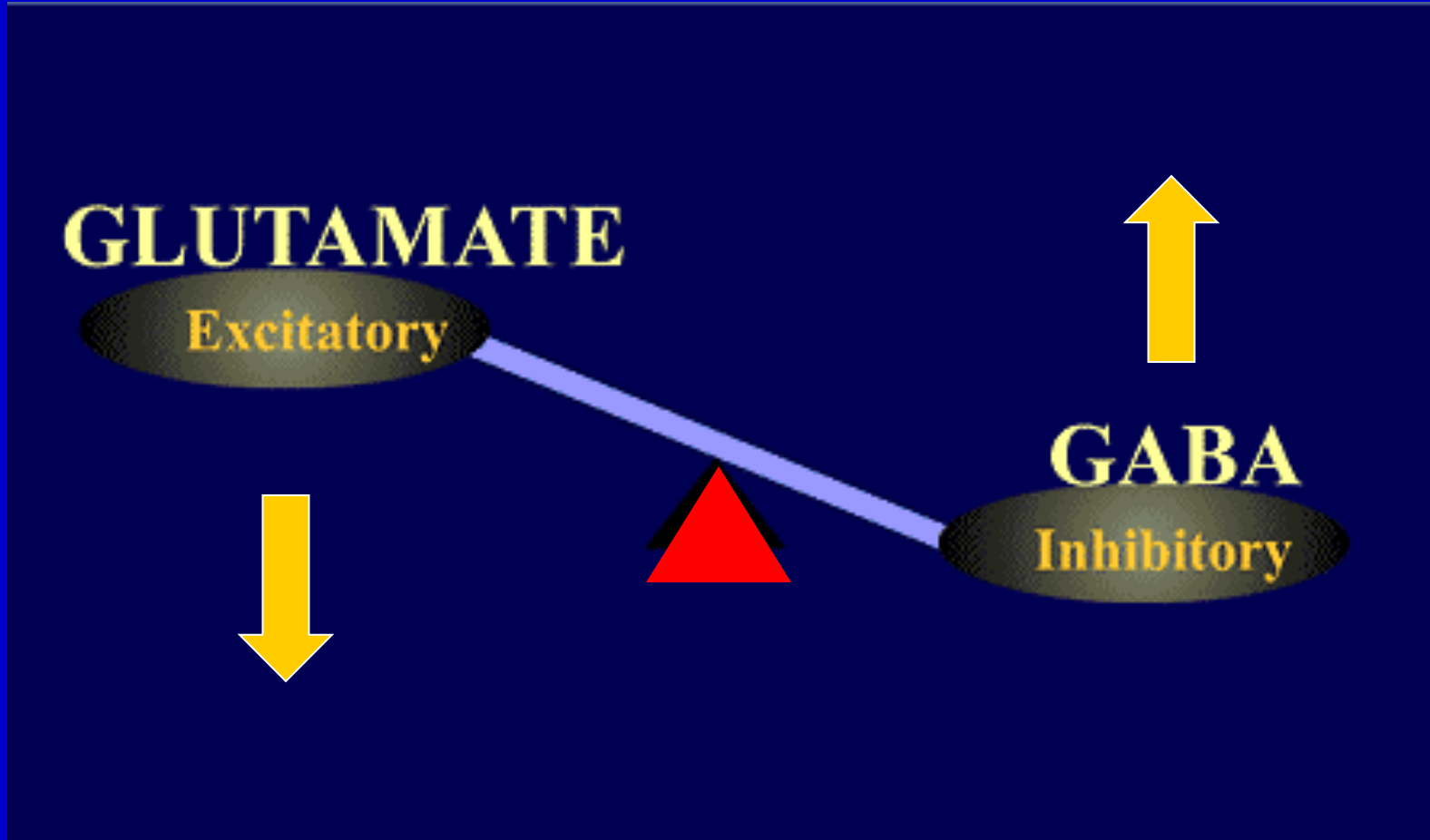


# Epileptogenesis



Epileptogenesis

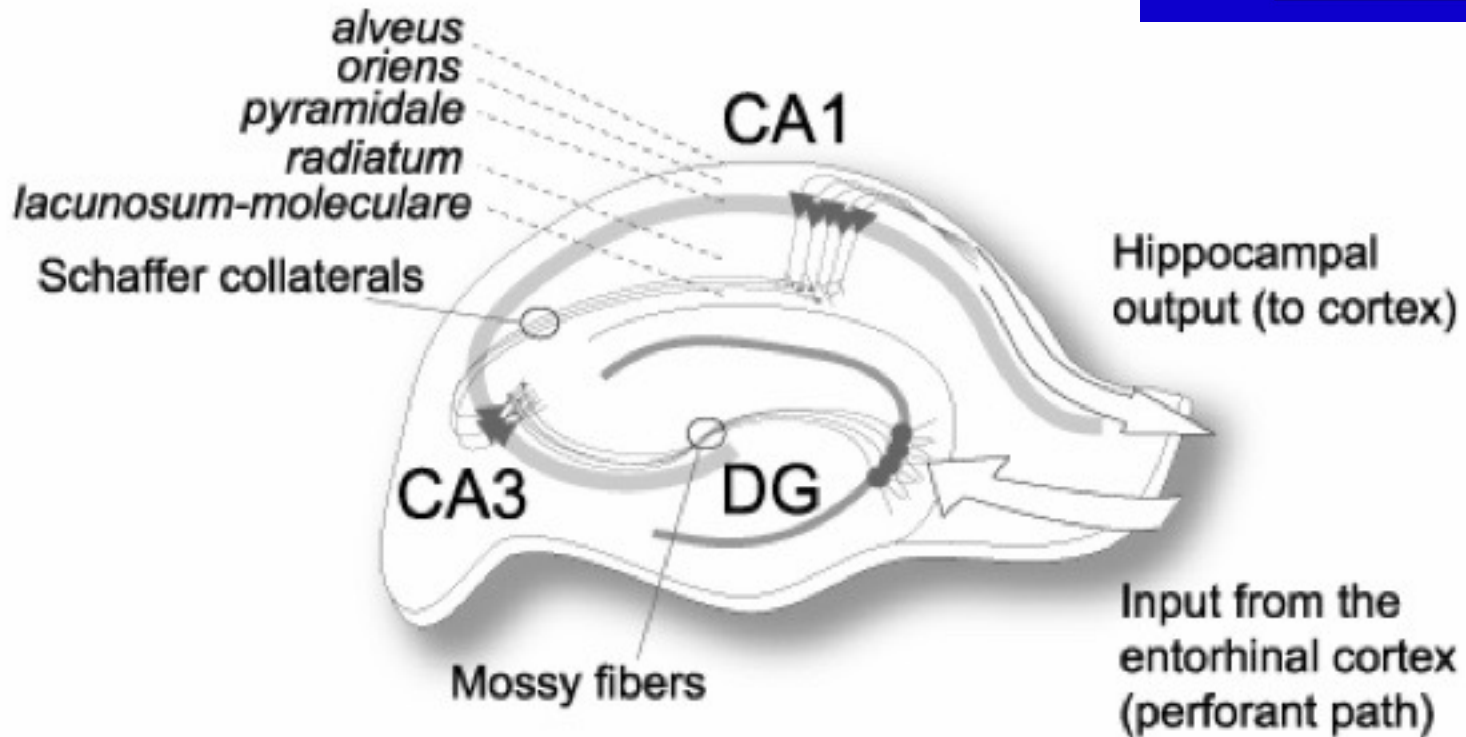
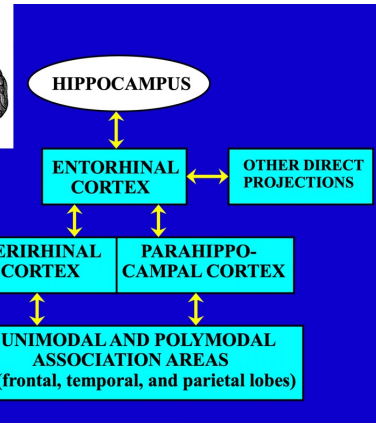
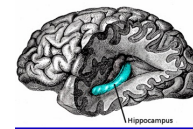
# Memory Deficits



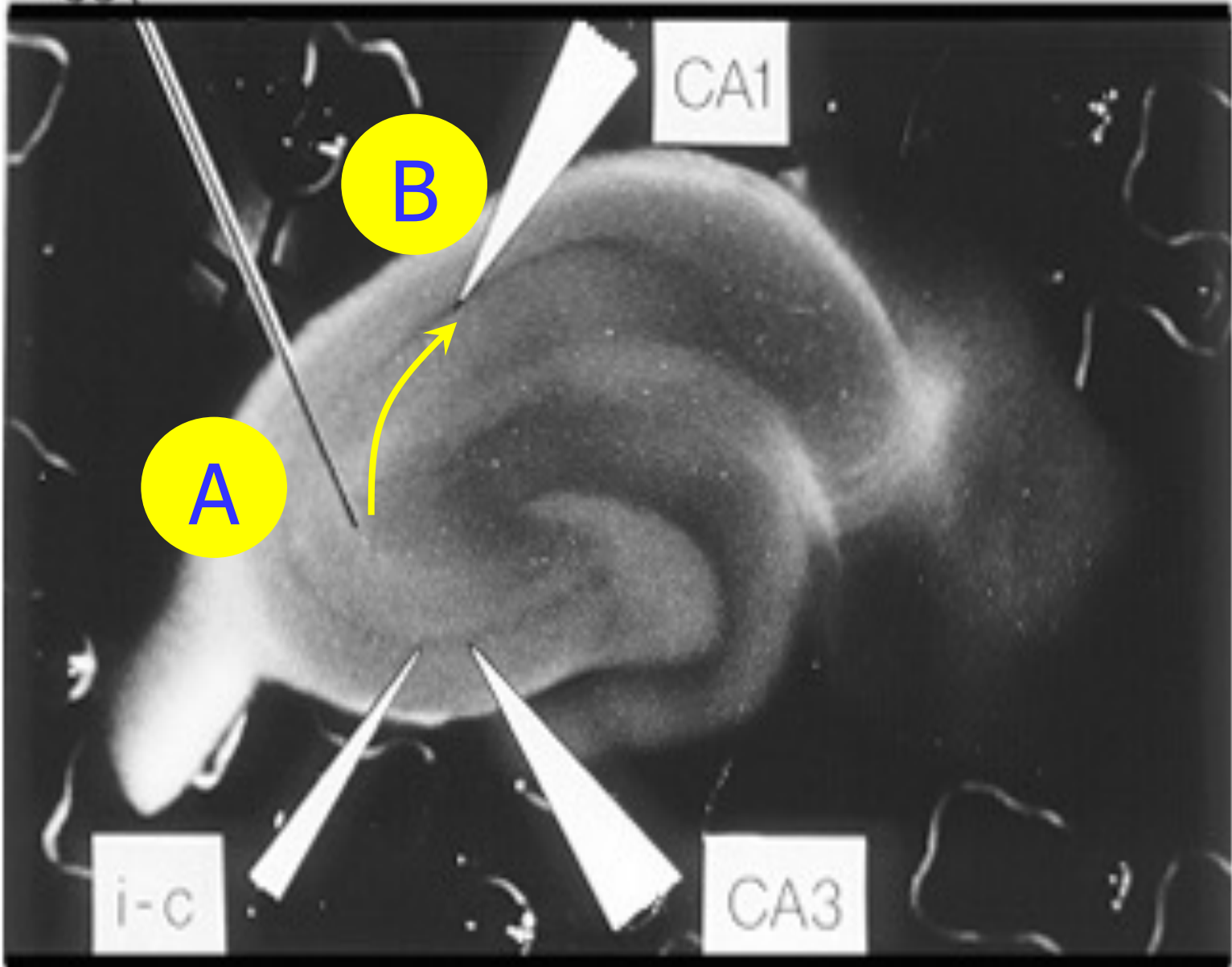
Memory Deficits

Is learning-related brain function affected differently by EtOH in adolescence and adulthood?

# Trisynaptic circuit in transverse hippocampal slice



STIM



CA1

B

A

i-c

CA3

# EtOH Effects on Memory

Behaving Animal

ANTEROGRADE MEMORY



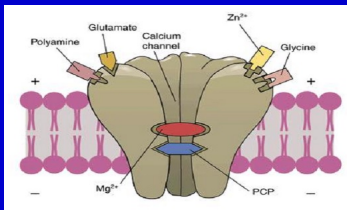
Neural Circuits

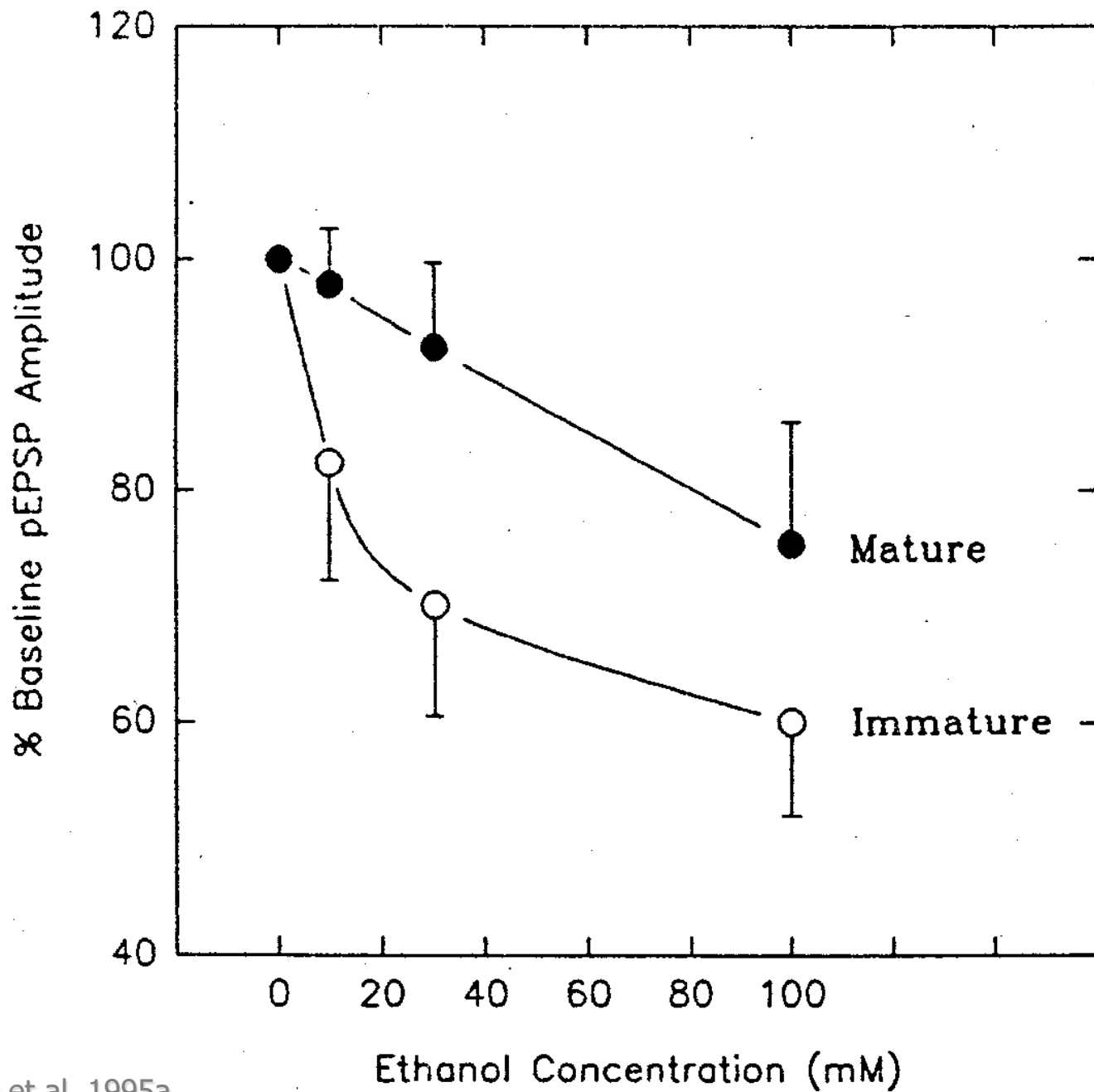
LONG-TERM POTENTIATION



Neuronal Membrane

**NMDA NEUROTRANSMISSION**

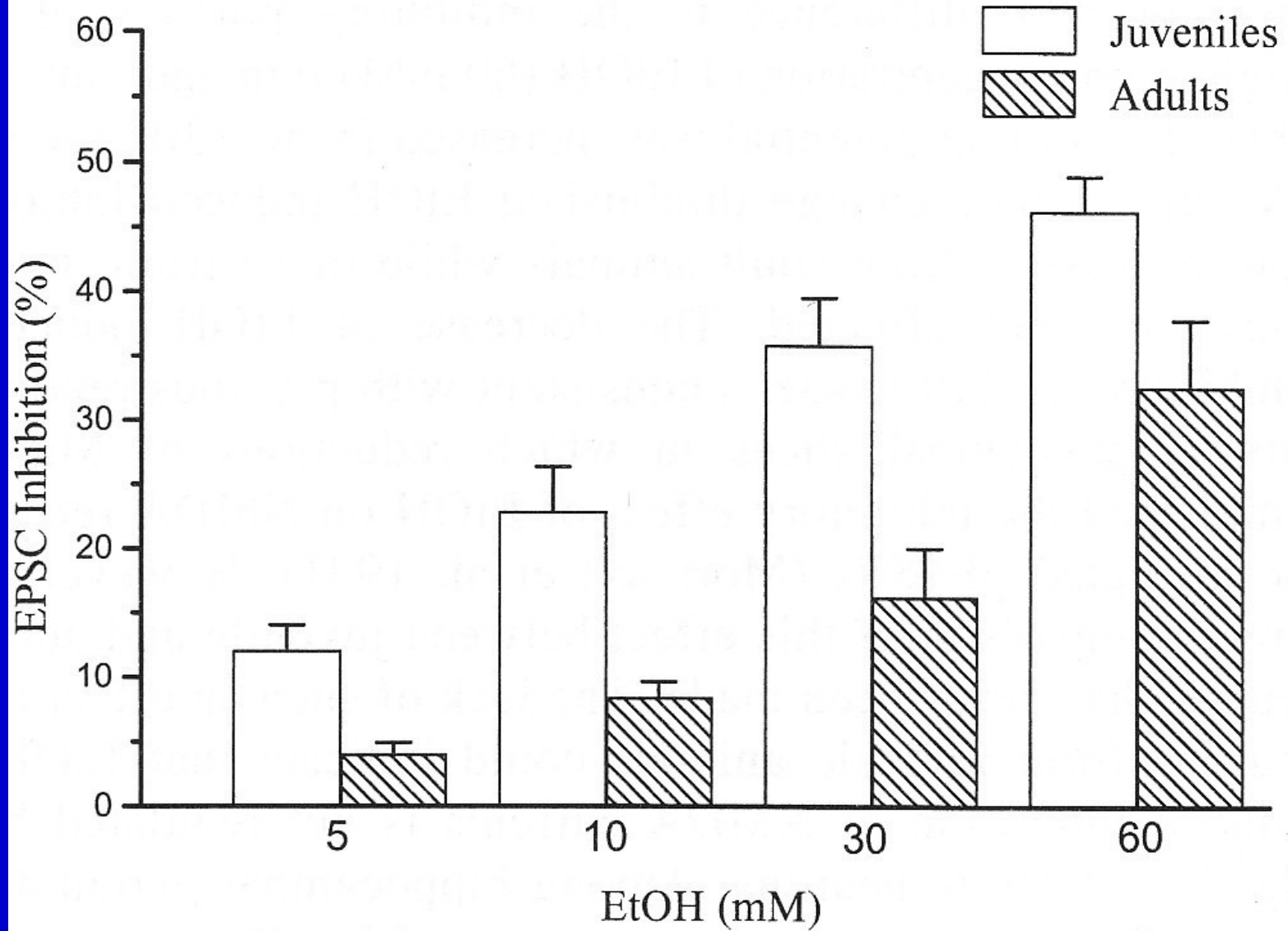




# Pyramidal Cell







# EtOH Effects on Memory

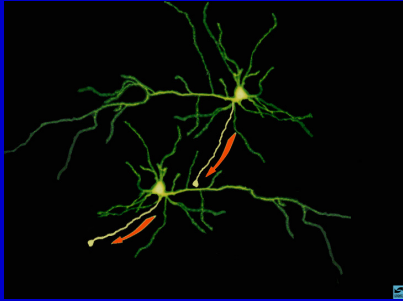
Behaving Animal

ANTEROGRADE MEMORY



Neural Circuits

**LONG-TERM POTENTIATION**

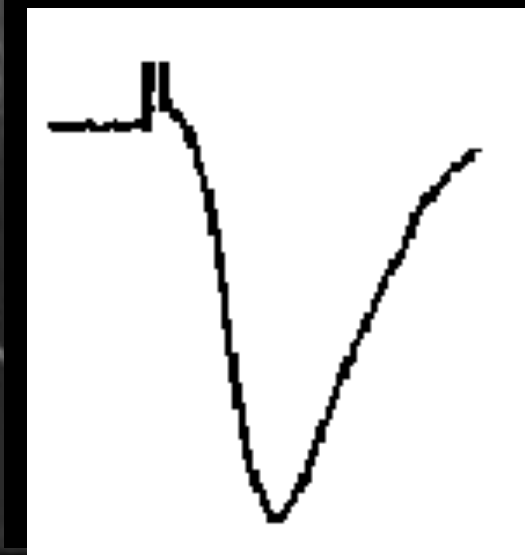
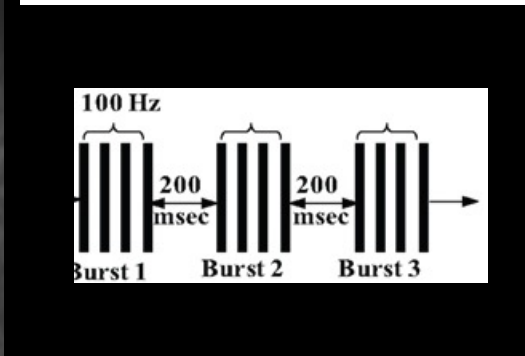
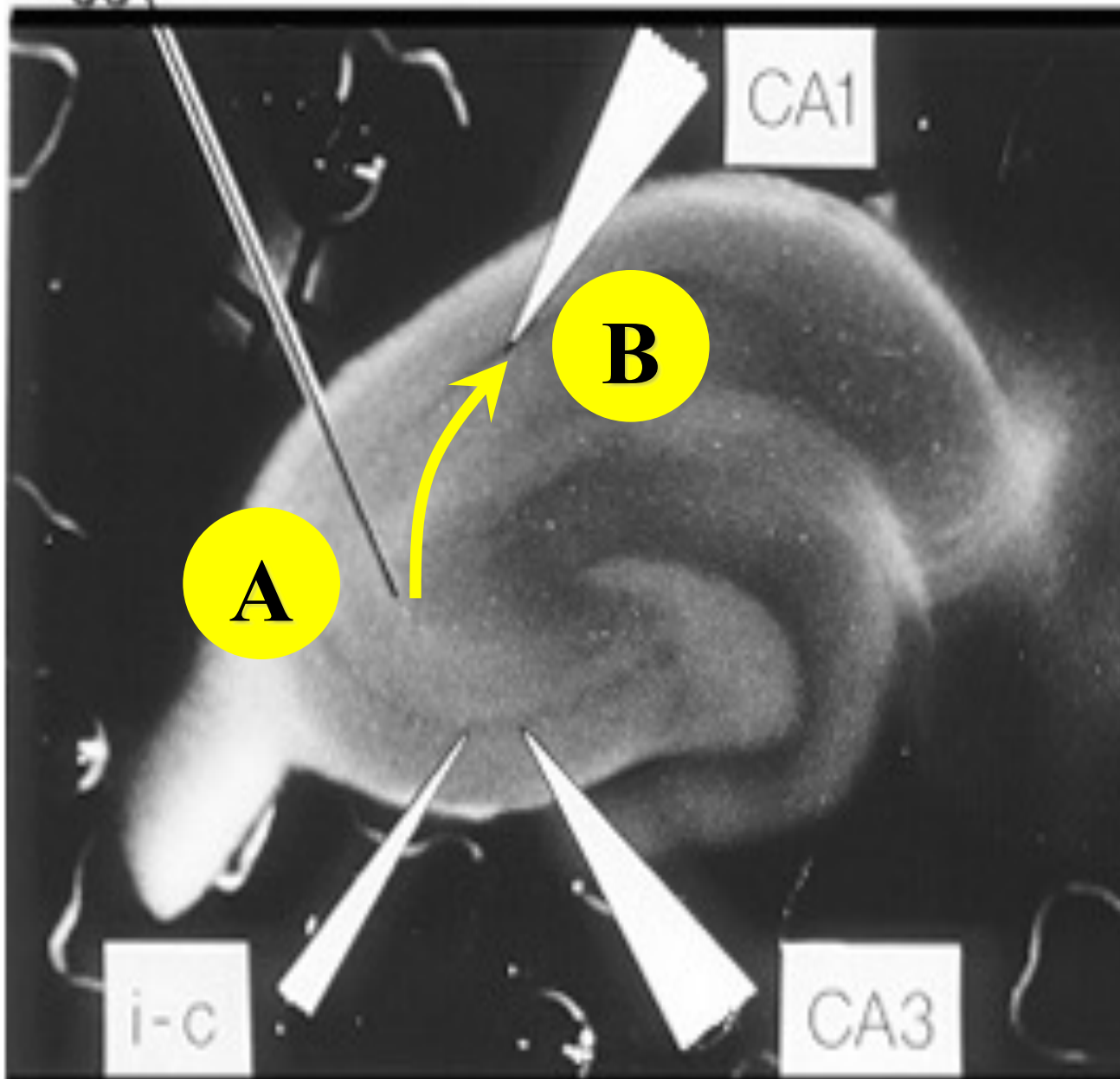


Neuronal Membrane

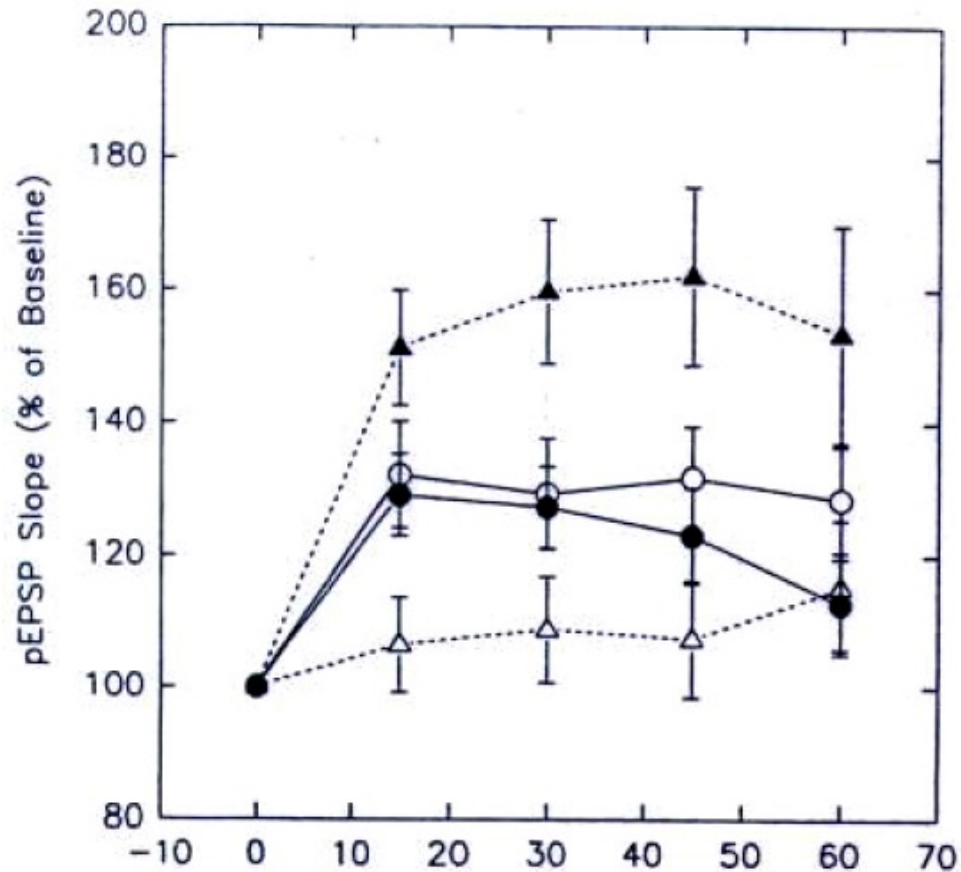
NMDA NEUROTRANSMISSION

STIM

# Long-Term Potentiation

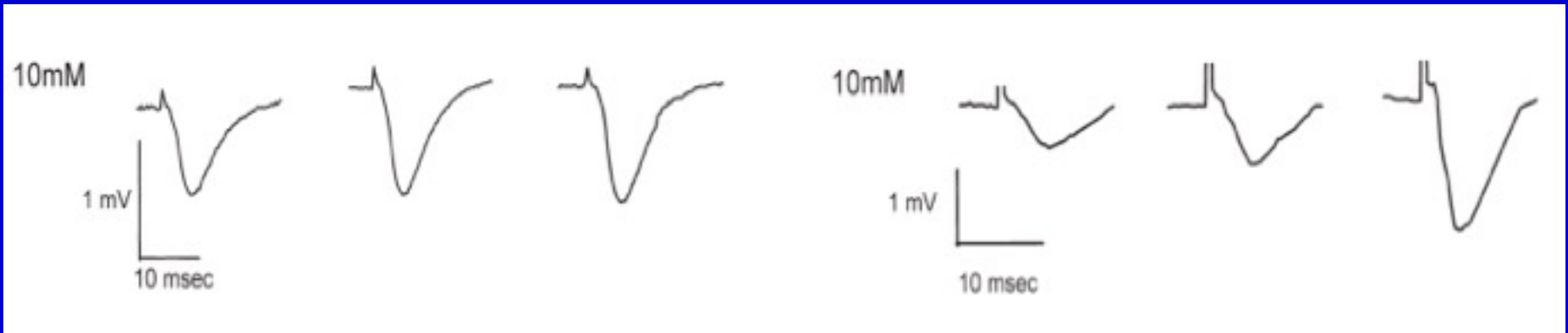


# Acute EtOH and LTP Induction in CA1



# Adolescent

# Adult



# Neural Mechanisms of Memory

Behaving Animal



**ANTEROGRADE MEMORY**



Neural Circuits

LONG-TERM POTENTIATION



Neuronal Membrane

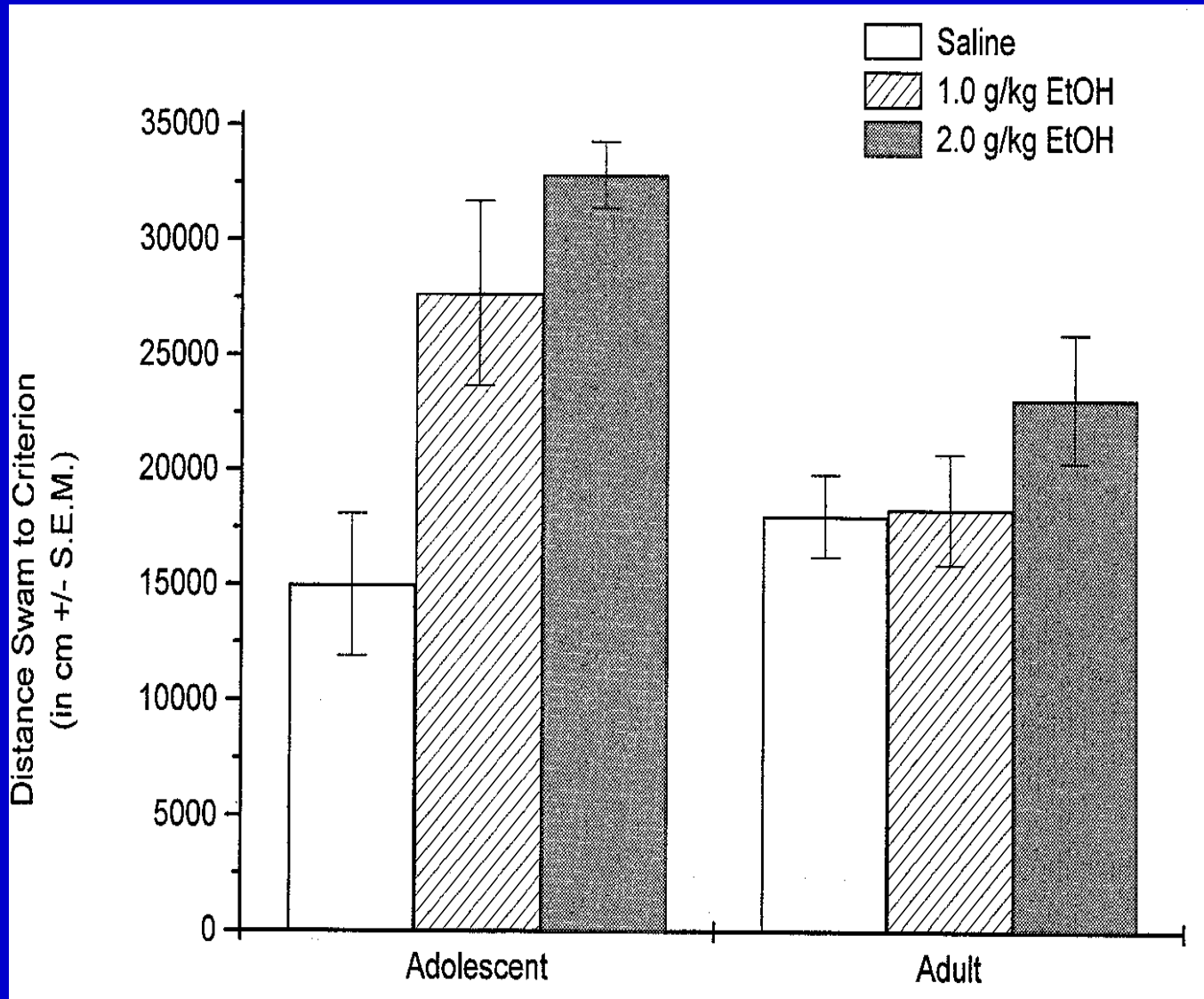
NMDA NEUROTRANSMISSION

# Morris Water Maze

## Spatial Learning and Memory



# Spatial Memory Acquisition





# Neural Mechanisms of Memory

Behaving Animal

**ANTEROGRADE MEMORY**



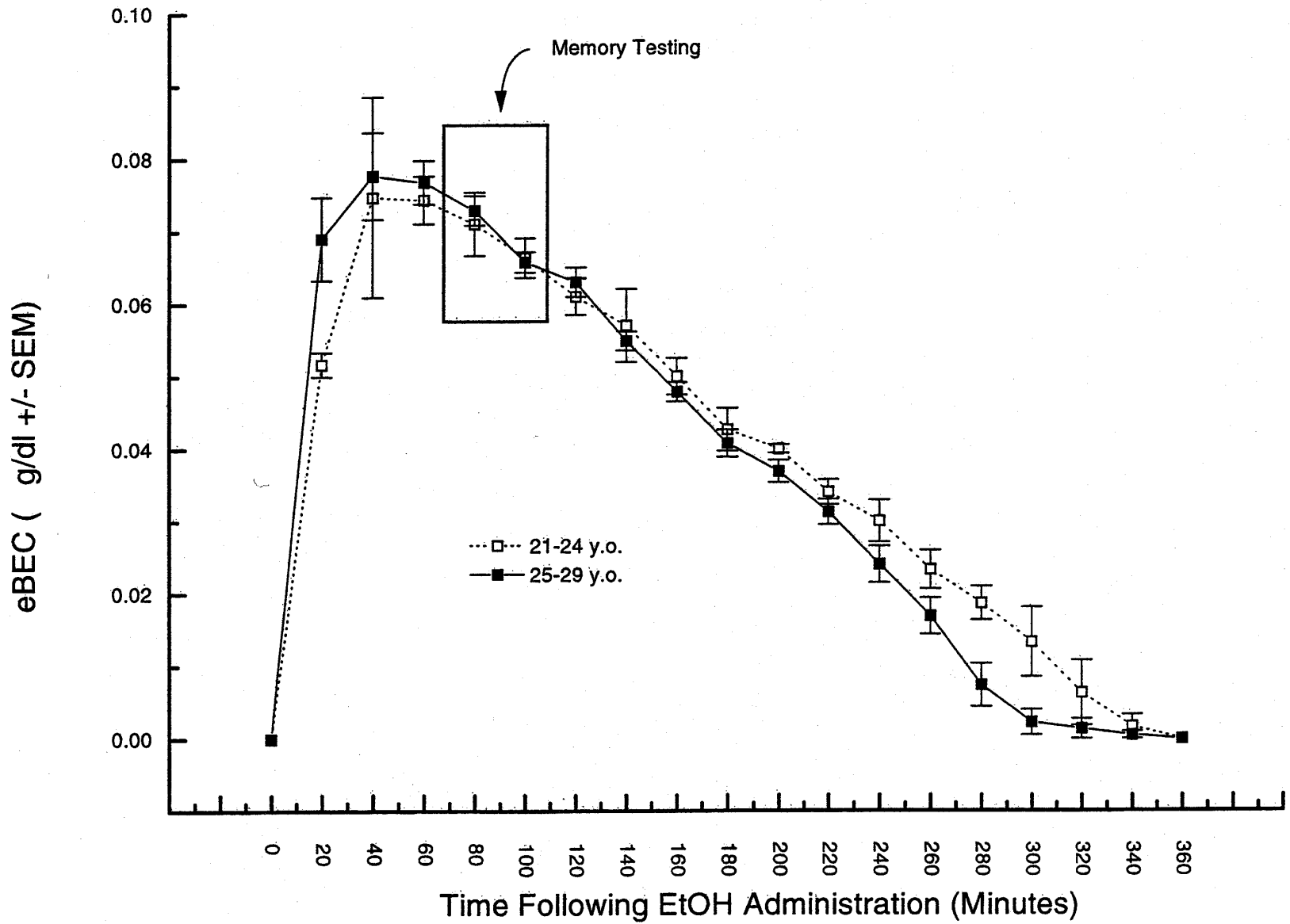
Neural Circuits

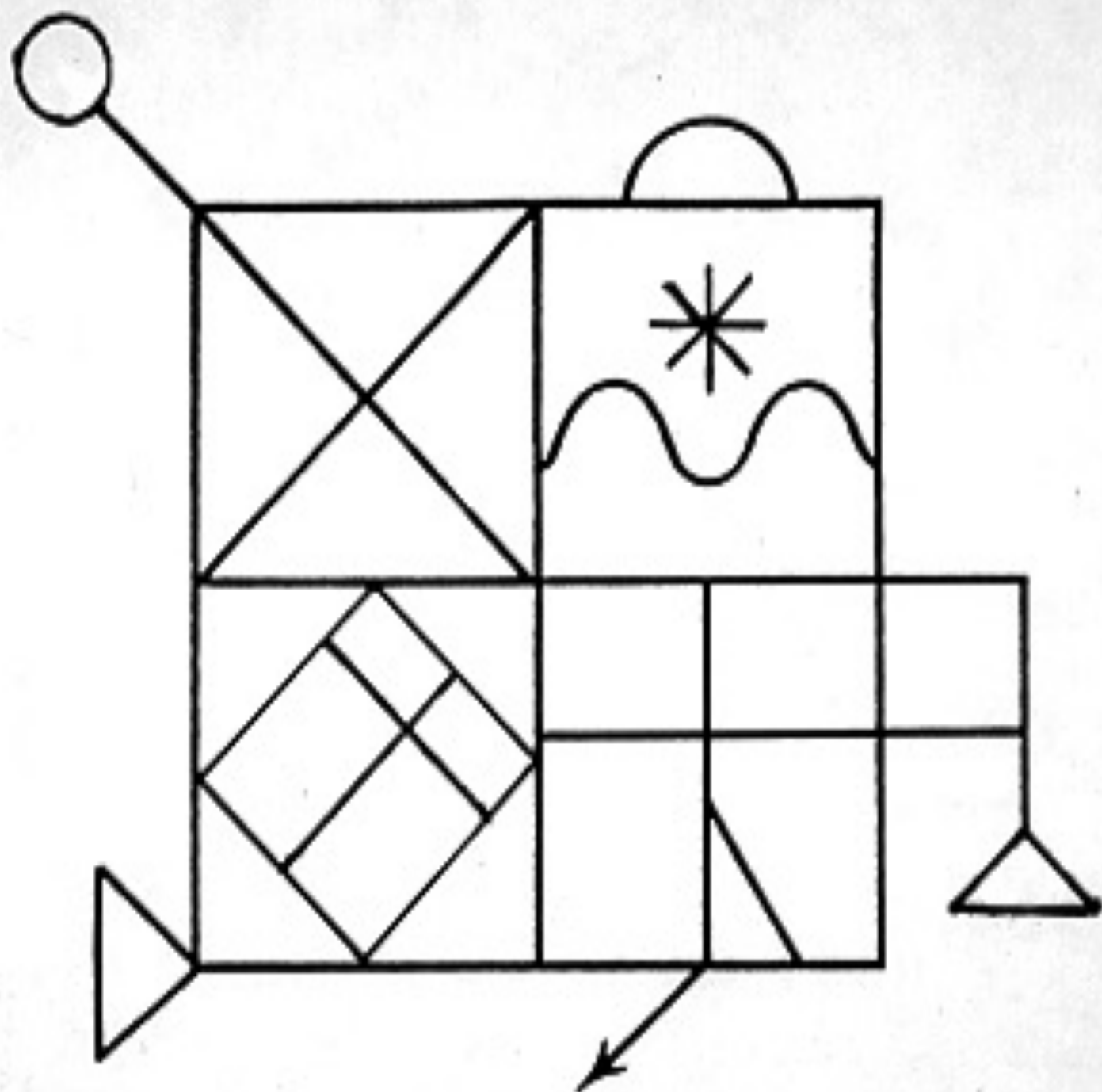
LONG-TERM POTENTIATION

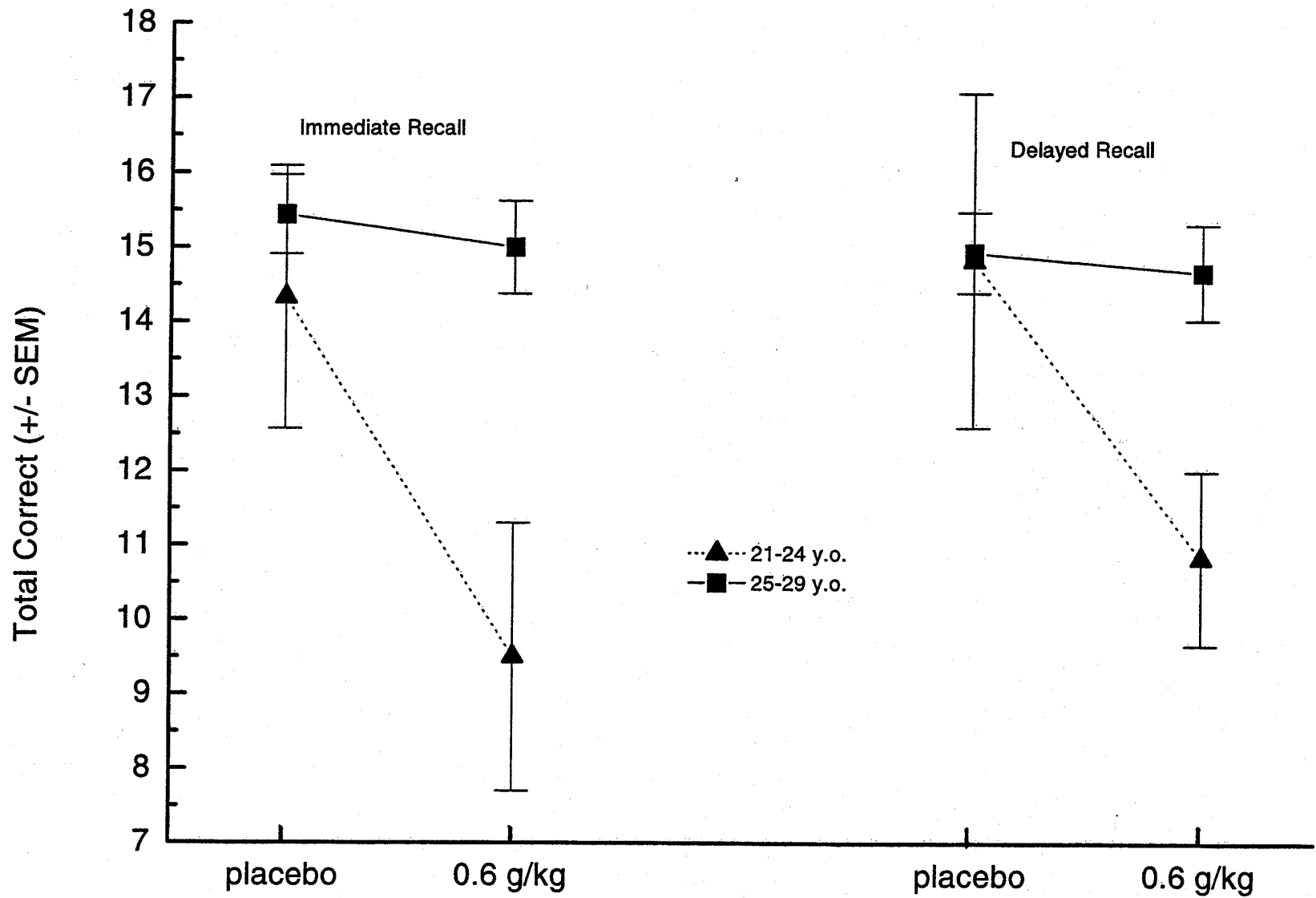
Neuronal Membrane

NMDA NEUROTRANSMISSION









# Acute Ethanol Effects

**Adult**

**Adolescent**

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**Memory-related synaptic signals**



**Memory-related circuit changes**



**Spatial Memory Acquisition**



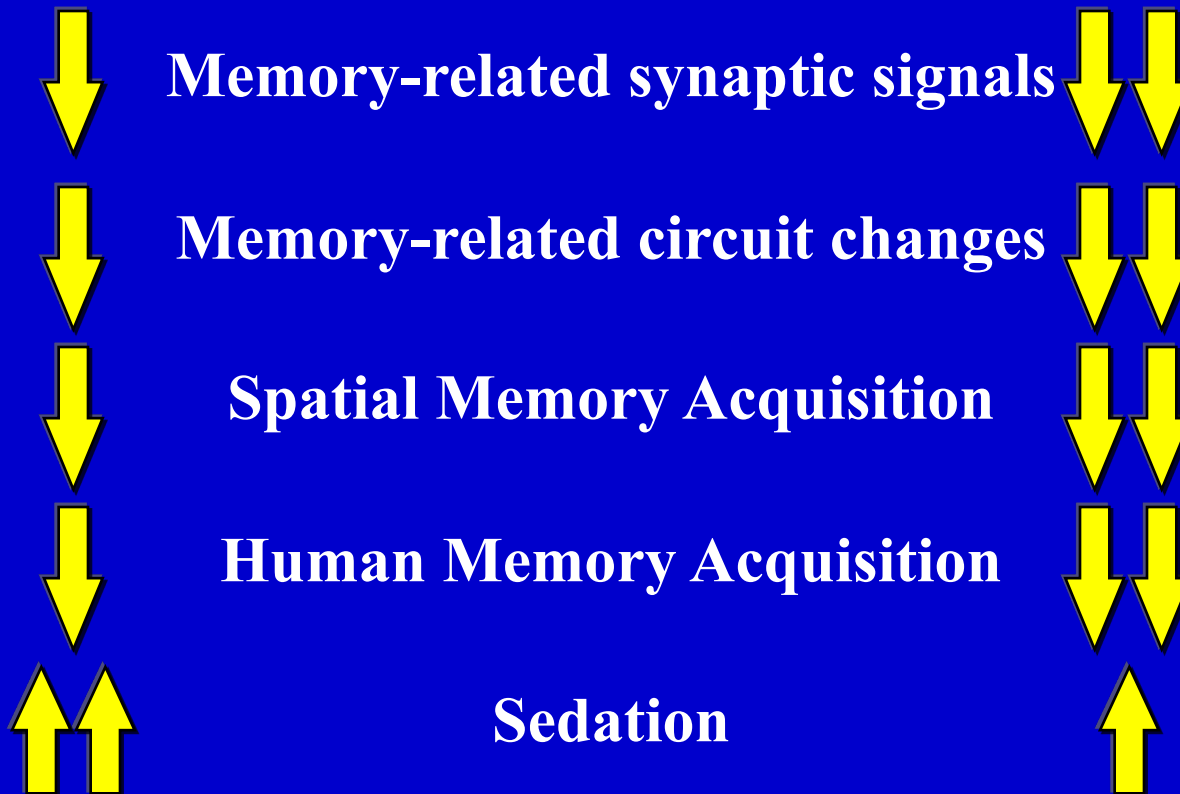
**Human Memory Acquisition**



# Acute Ethanol Effects

Adult

Adolescent



# Alcohol and Sedation

Adolescent



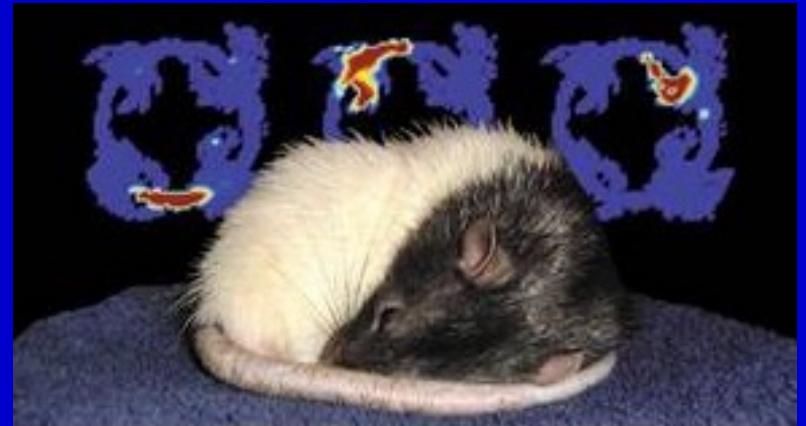
Adult



# Alcohol and Sedation

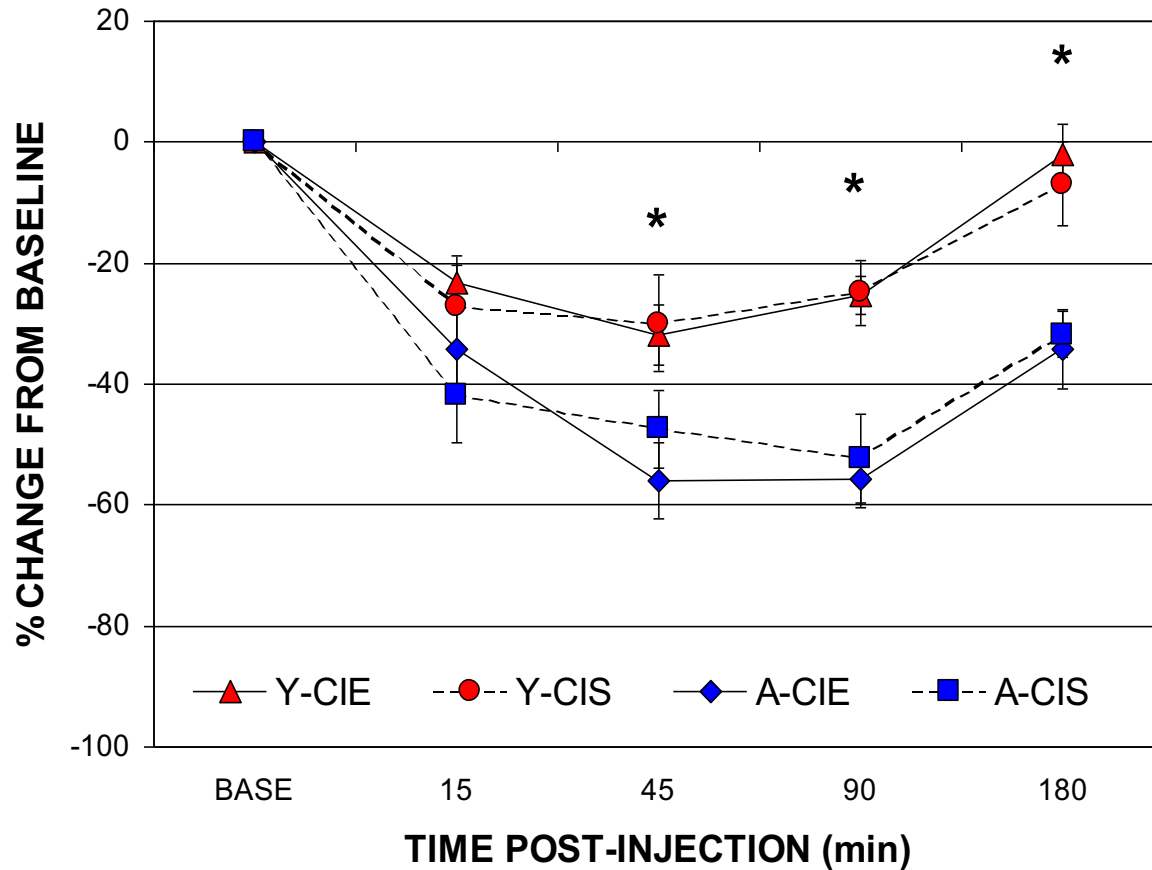
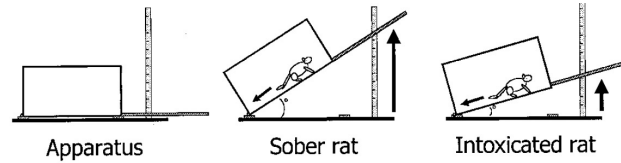
Adolescent

Adult





# Acute EtOH Effect on Tilt-Plane Performance



Similar effects on sleep time and intoxication scores

Due to developmental differences in the potency of EtOH on learning and sedation, adolescents are:

- *Less* likely to feel impaired
- *More* likely to be impaired

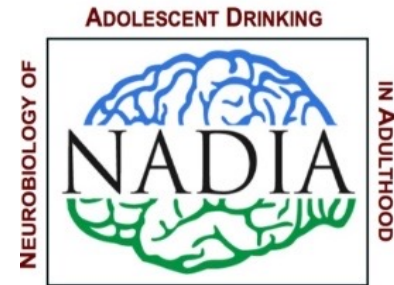
What are the long-term effects of drinking during adolescence?

Is the adolescent brain more vulnerable to damage by alcohol?

Can alcohol alter the normal trajectory brain development during adolescence?

# Many enduring effects of AIE have been identified, including:

- learning
- social behavior
- affective behavior
- circadian function
- synaptic function and plasticity
- neuroimmune reactivity
- dendritic spine density/morphology
- epigenetic function
- glial function
- neurogenesis



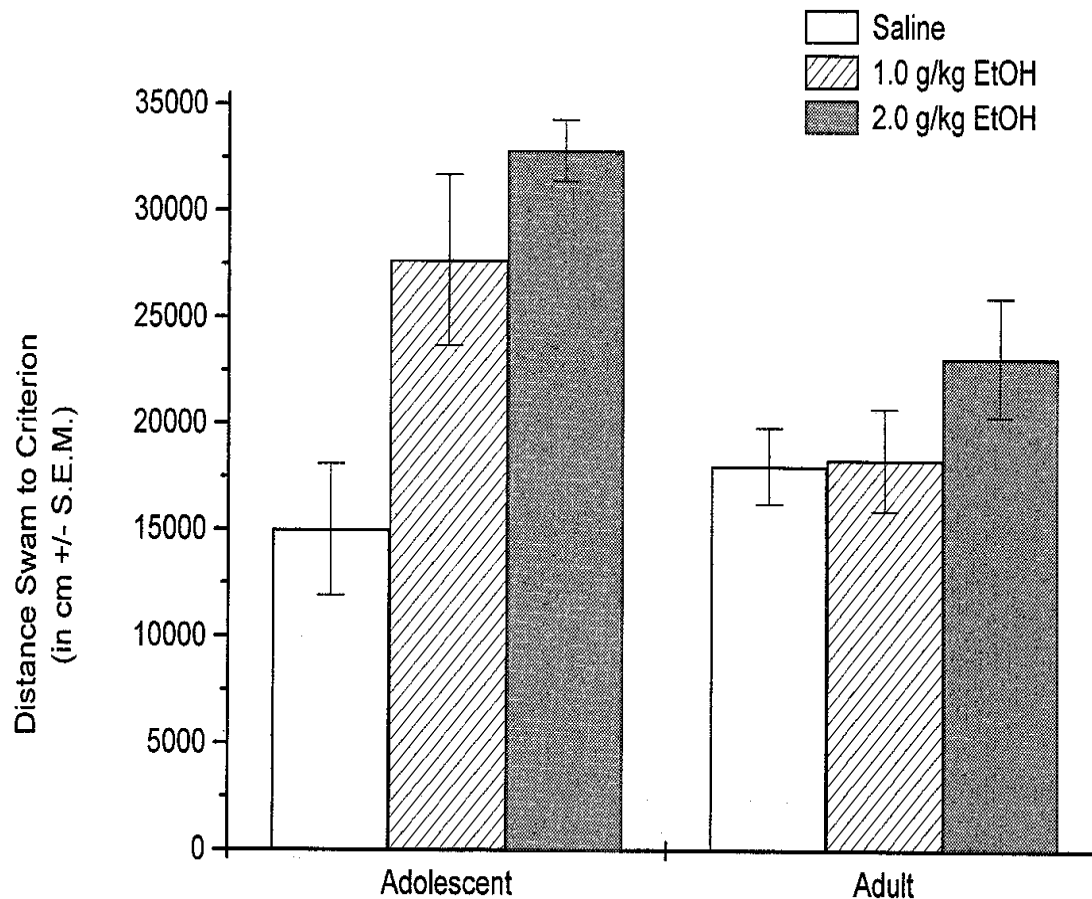
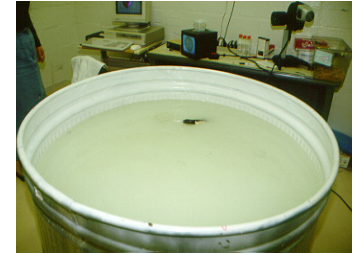
Convergence within findings: Apparent retention of adolescent-typical phenotypes in adulthood, after AIE

As if adolescent characteristics are “Locked-In” by the alcohol exposure

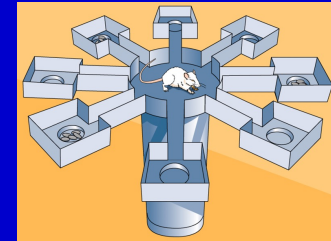
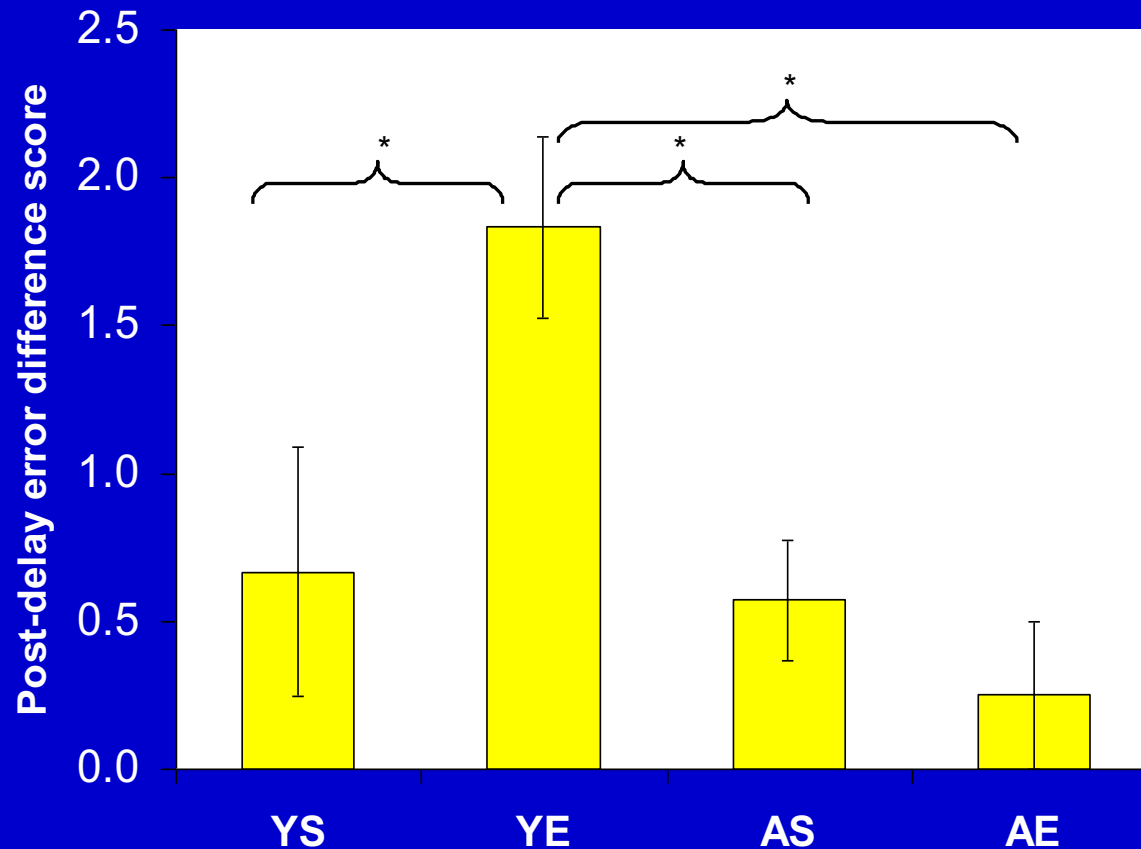
# Lock-In

Behavioral Sensitivity to Acute Ethanol

# Adolescent rats are more sensitive to ethanol-induced memory impairment than adults



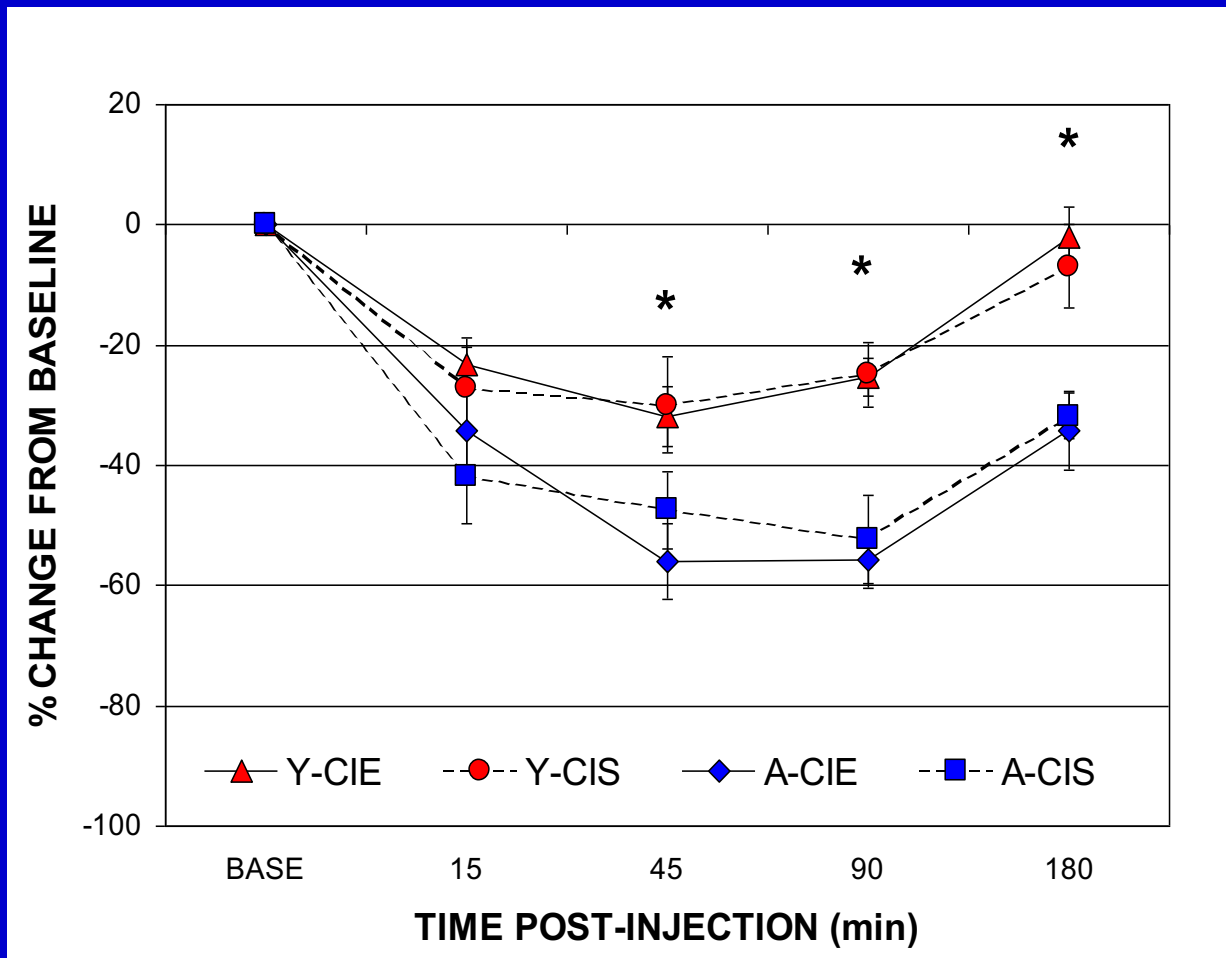
...and adult rats pre-exposed to AIE remain highly sensitive to the effects of acute EtOH on memory



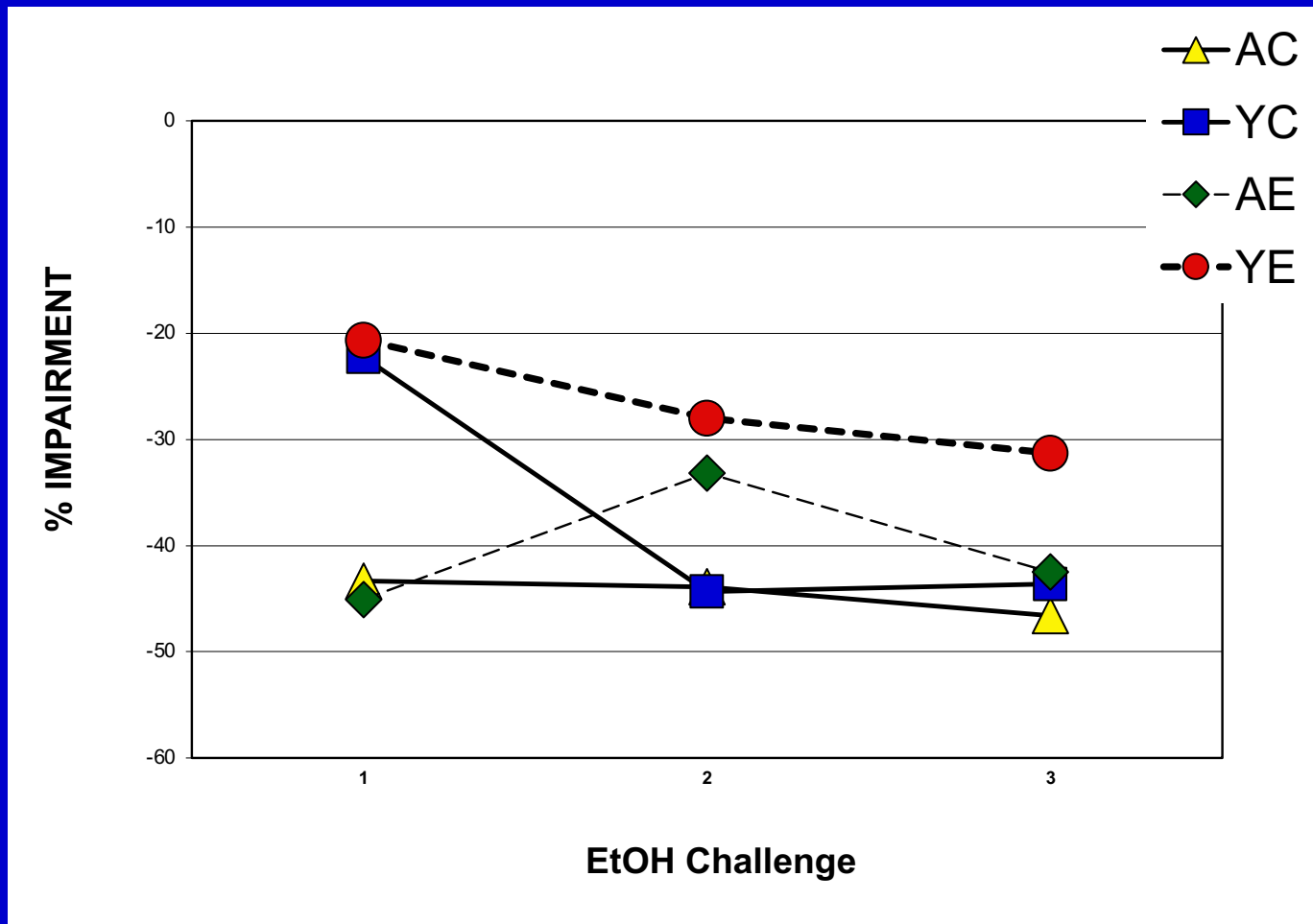
But repeated EtOH exposure in adulthood did not lead to greater EtOH sensitivity later



# Adolescent rats are less sensitive to ethanol-induced motor impairment than adults



# Adult rats pre-exposed to AIE retain low sensitivity to the effects of acute ethanol on motor function



# Lock-In

## Maintenance of Behavioral Sensitivity to Acute Ethanol

- Less sensitivity to EtOH-induced CTA
- Less sensitivity to EtOH-induced chrono-disruption (males)
- Less sensitivity to impairment of social behavior with high doses
- More sensitivity to facilitation of social behavior with low doses
- Less anxiety during ethanol withdrawal
- More sensitive to the rewarding effects of ethanol
- More voluntary ethanol consumption

Alaux-Cantin et al., 2013  
Mejia-Tiober et al., 2014

Varlinskaya et al., 2014  
Ruby et al, 2017; 2018

# Lock-In

## Maintenance of Adolescent-Typical Baseline Behavioral Characteristics

- Disinhibition in open field
- Increased open arm exploration in EPM
- Potentiation of context fear conditioning by paired CS<sup>+</sup> (paired CS<sup>+</sup> reduces fear conditioning in normal adults).

Ehlers et al., 2013

Gilpin et al., 2012

Broadwater and Spear, 2014

Ruby et al, 2017; 2018

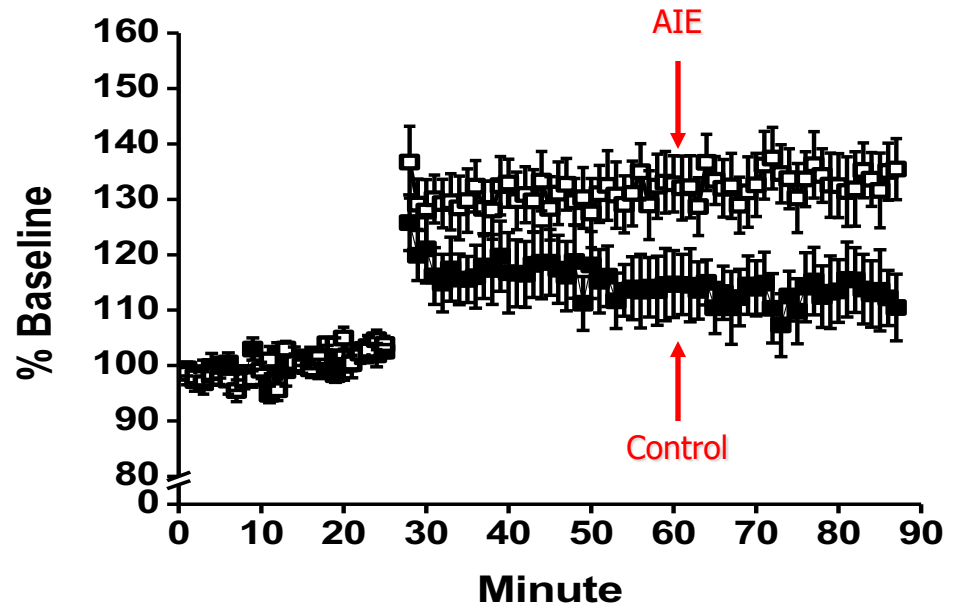
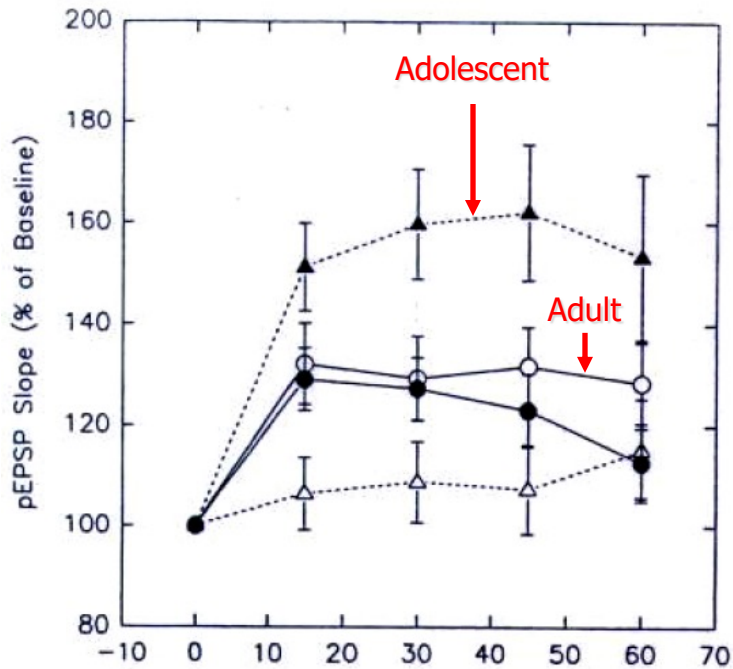
# Lock-In

Maintenance of Adolescent-Typical  
Synaptic Function

# Lock-In

## Maintenance of Adolescent-Typical Synaptic Plasticity

### LTP



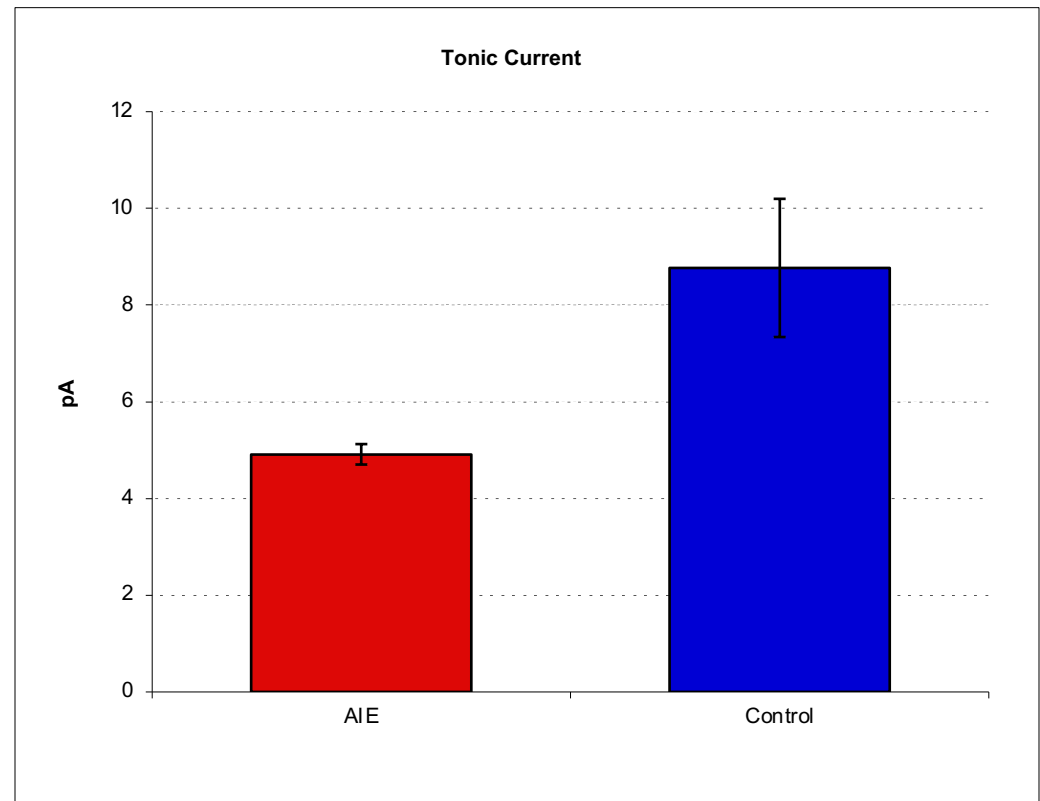
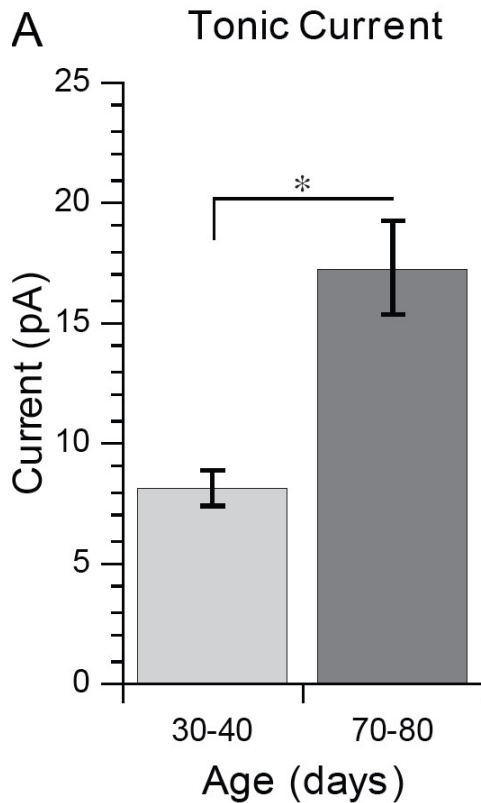
LTP of greater amplitude during adolescence

...and in adulthood after AIE

# Lock-In

## Maintenance of Adolescent-Typical GABA Function

### GABA<sub>A</sub>R-Mediated Tonic Current



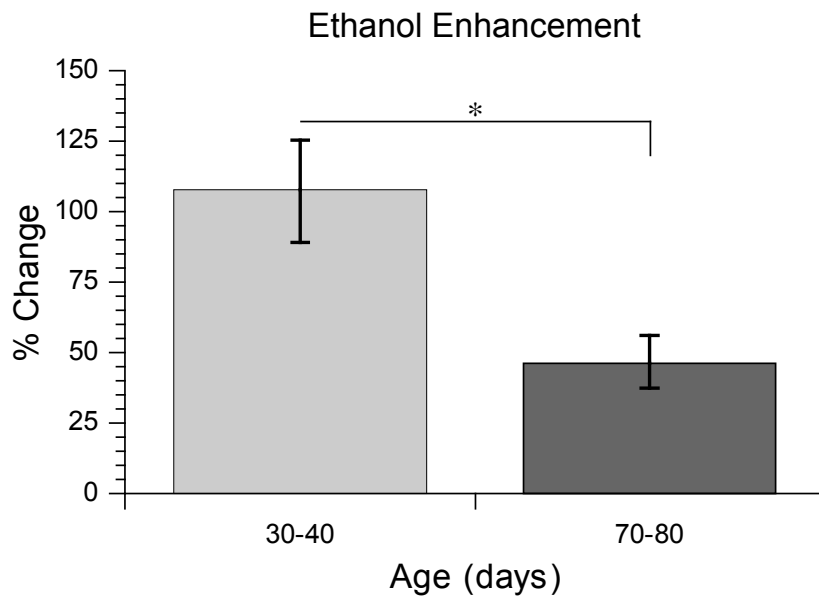
Tonic current lower during adolescence

...and in adulthood after AIE

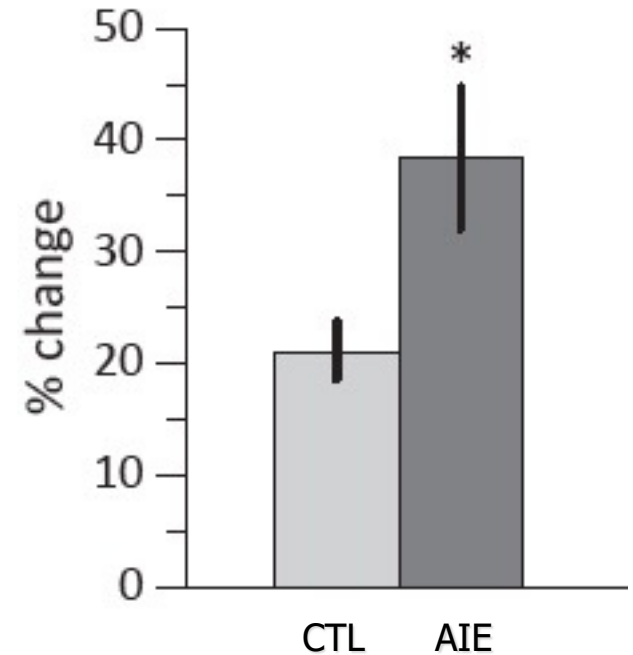
# Lock-In

## Maintenance of Adolescent-Typical GABA Function

### EtOH sensitivity of GABA<sub>A</sub>R-Mediated Tonic Current



Greater EtOH sensitivity during adolescence



...and in adulthood after AIE

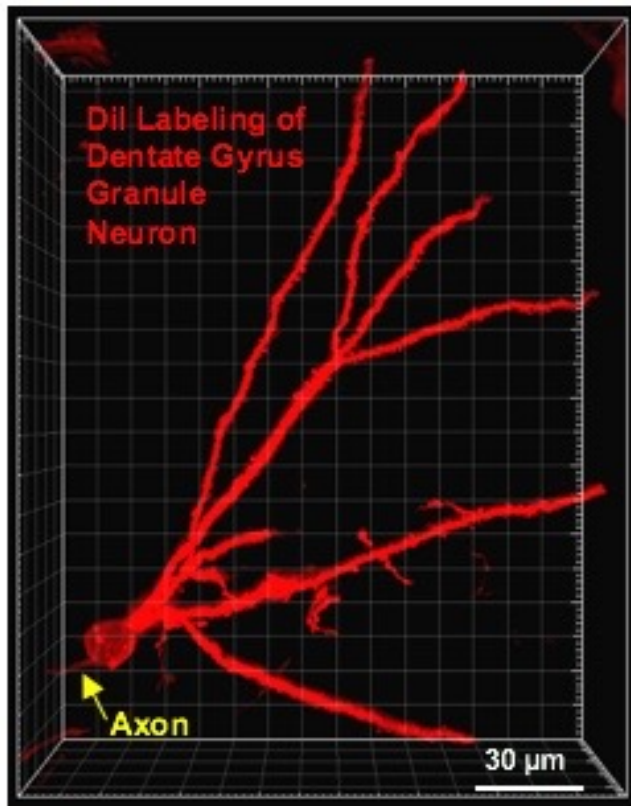


# Can AIE Effects be Reversed?

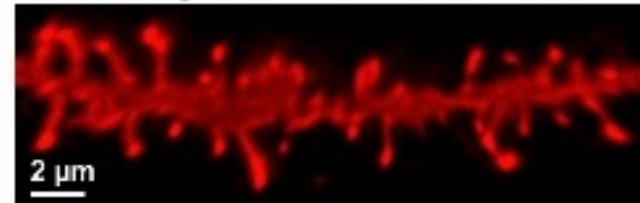
We have studied two drugs in common clinical use:

- Donepezil (Aricept)
- Gabapentin (Neurontin)

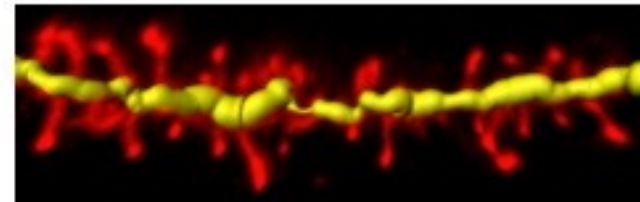
# Dendritic Spines on Dentate Granule Cells



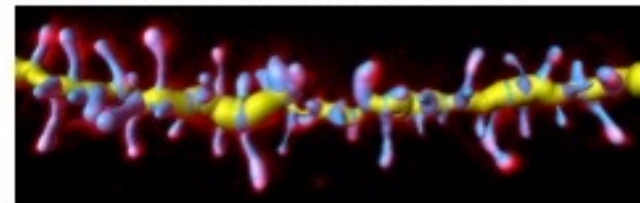
Dil Labeling



Imaris Filament of Dendritic Shaft

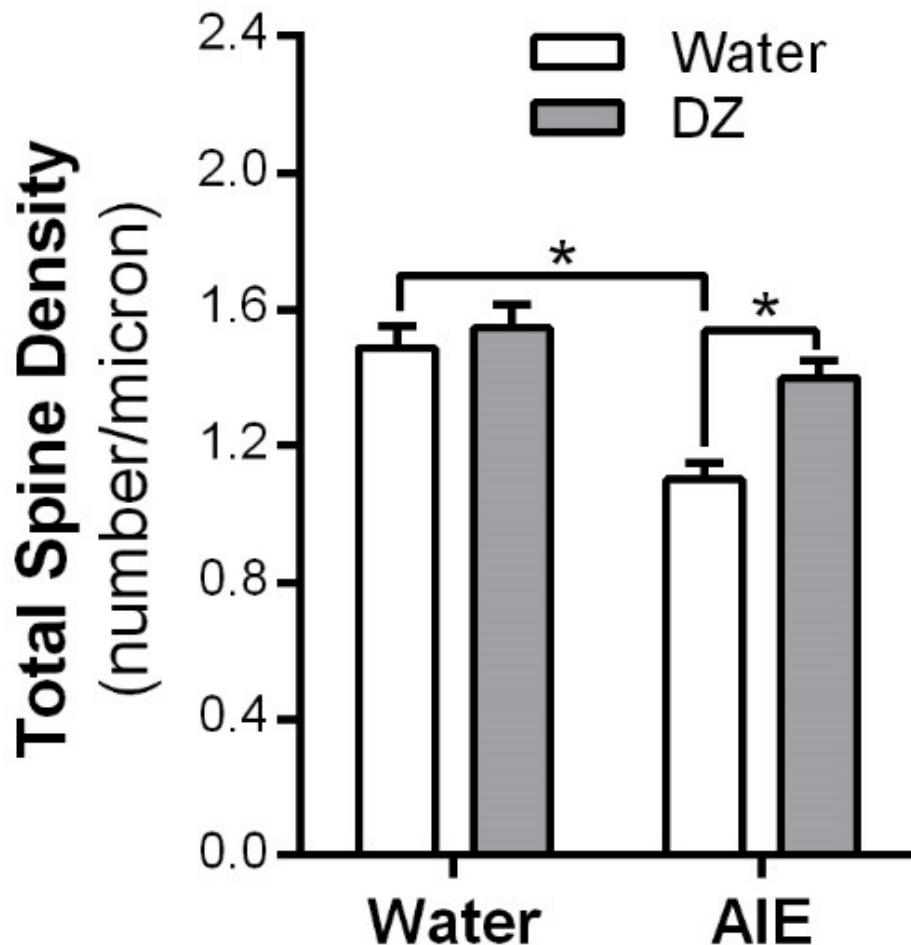


Imaris Filament of Shaft & Spines

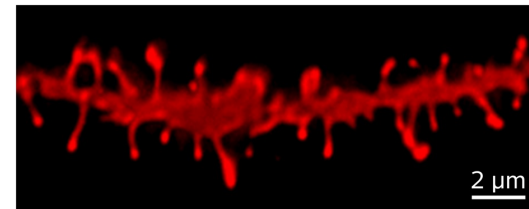


# Dendritic Spines on HPC Dentate Granule Cells

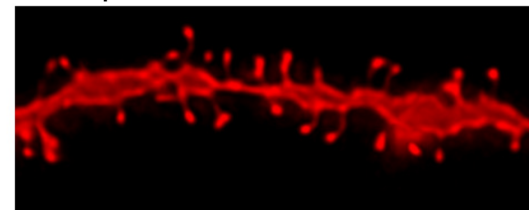
- Reduced by AIE
- Restored by sub-chronic Donepezil



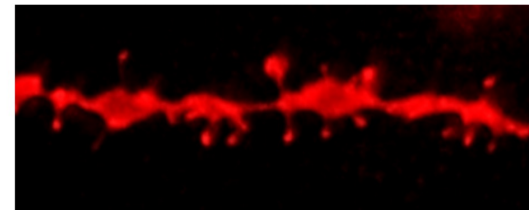
Control



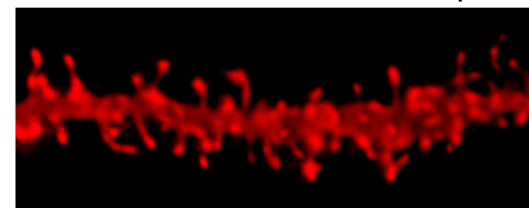
Donepezil



Adolescent Alcohol

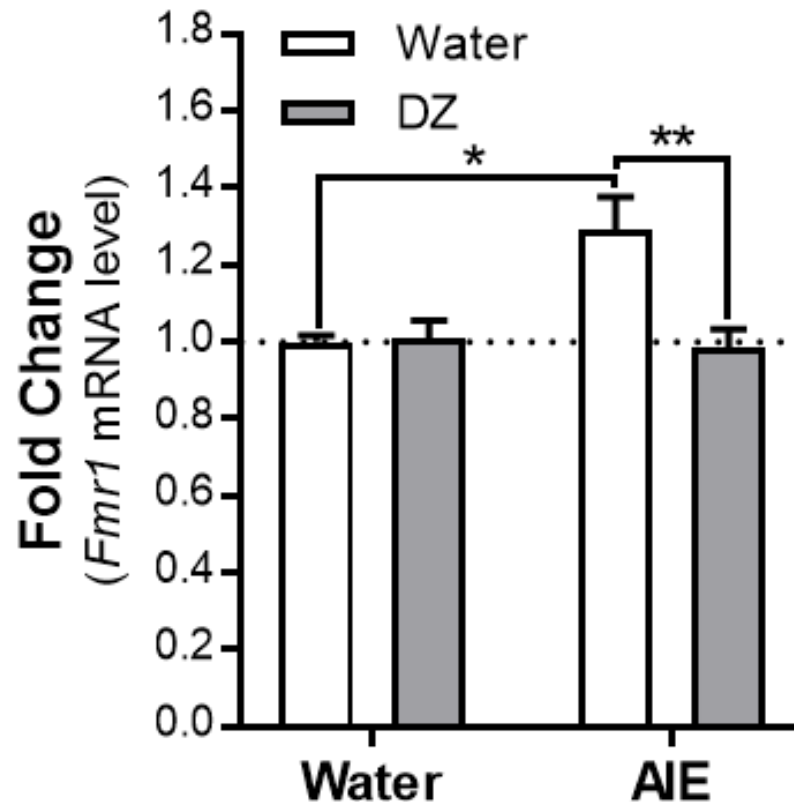


Adolescent Alcohol + Donepezil



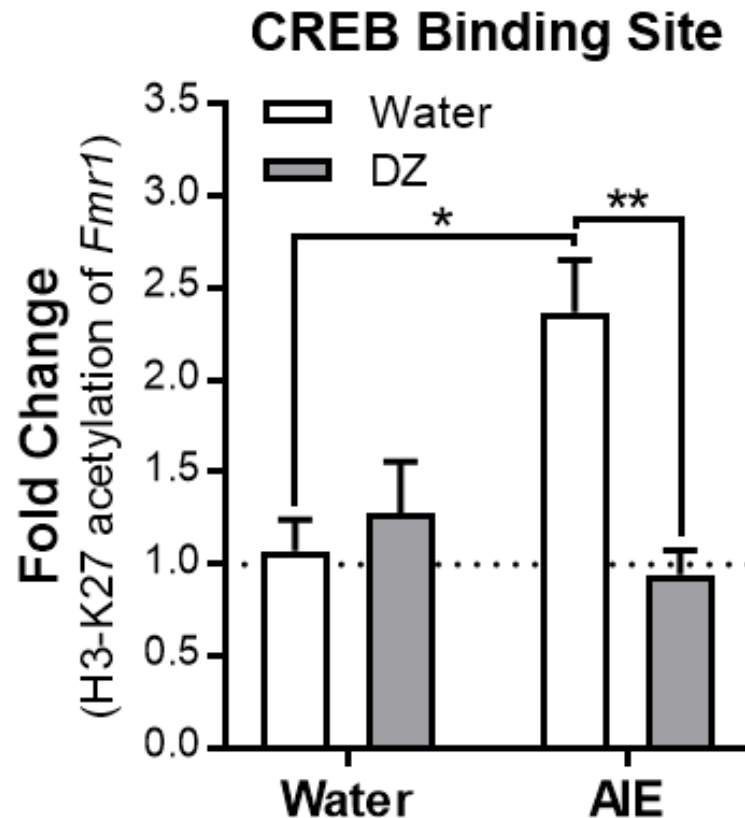
# *Fmr1* gene expression is known to regulate spine structure and density

- Increased by AIE
- Restored by sub-chronic Donepezil in adulthood



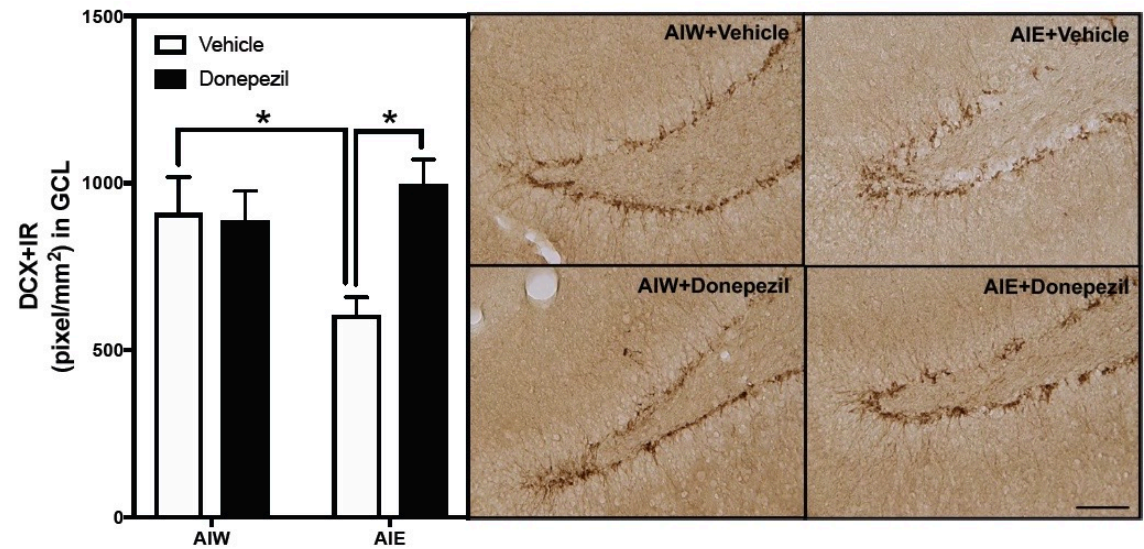
# Epigenetic Regulation of *Fmr1*

- Increased by AIE (→ gene overexpression)
- Restored by sub-chronic Donepezil in adulthood

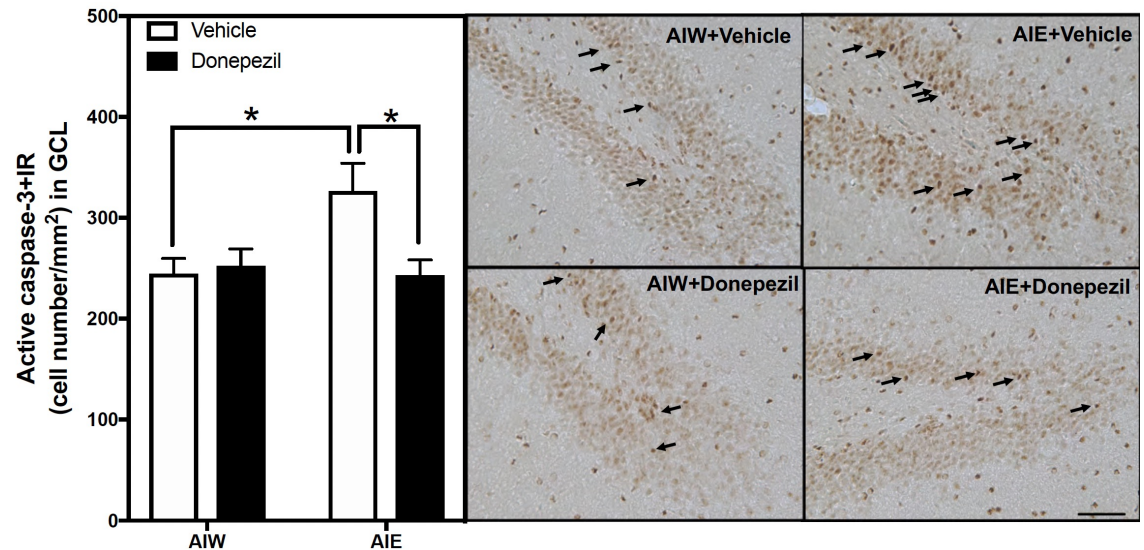


# Neurogenesis and Cell Death Markers

AIE decreases HPC neurogenesis – reversed by donepezil

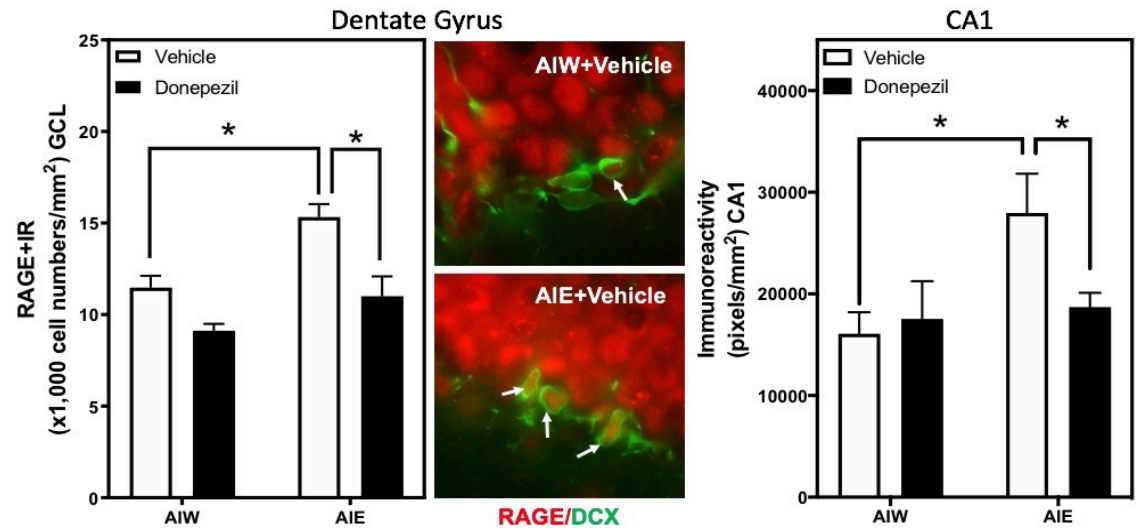


AIE increases HPC cell death markers – reversed by donepezil

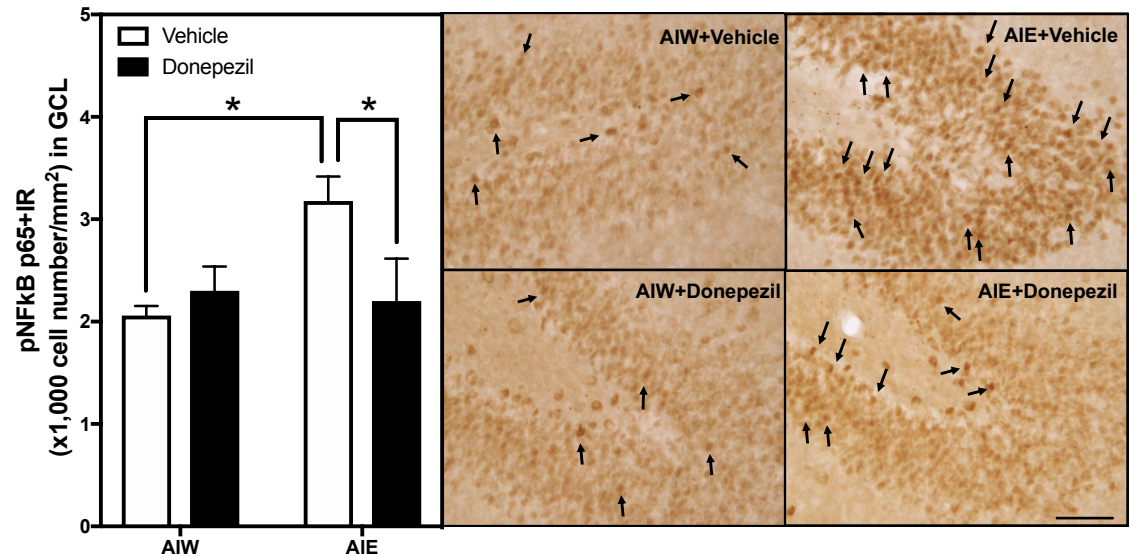


# Neuroimmune Signaling Markers

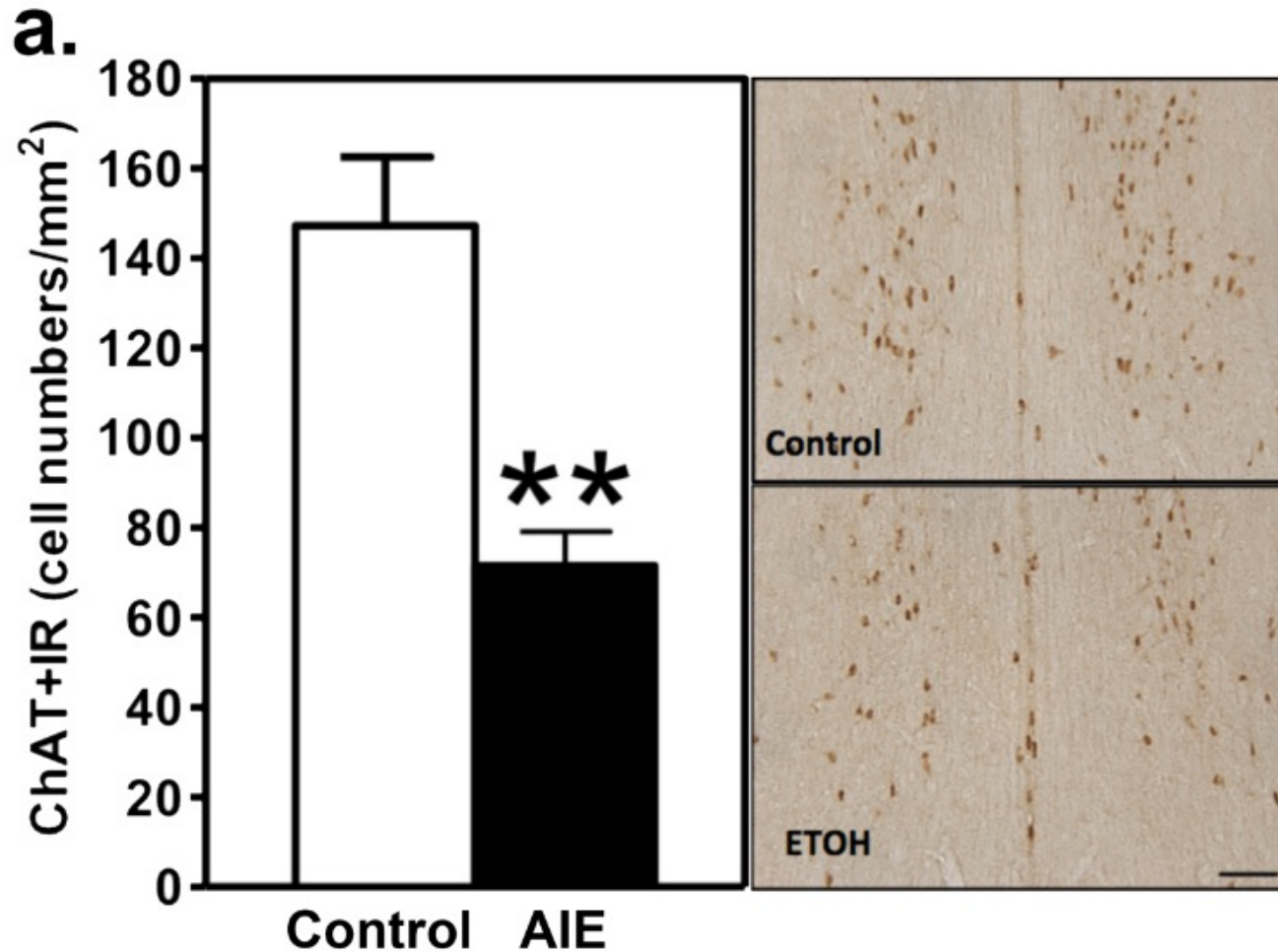
AIE increases HPC RAGE –  
reversed by donepezil



AIE increases HPC pNfKB  
reversed by donepezil

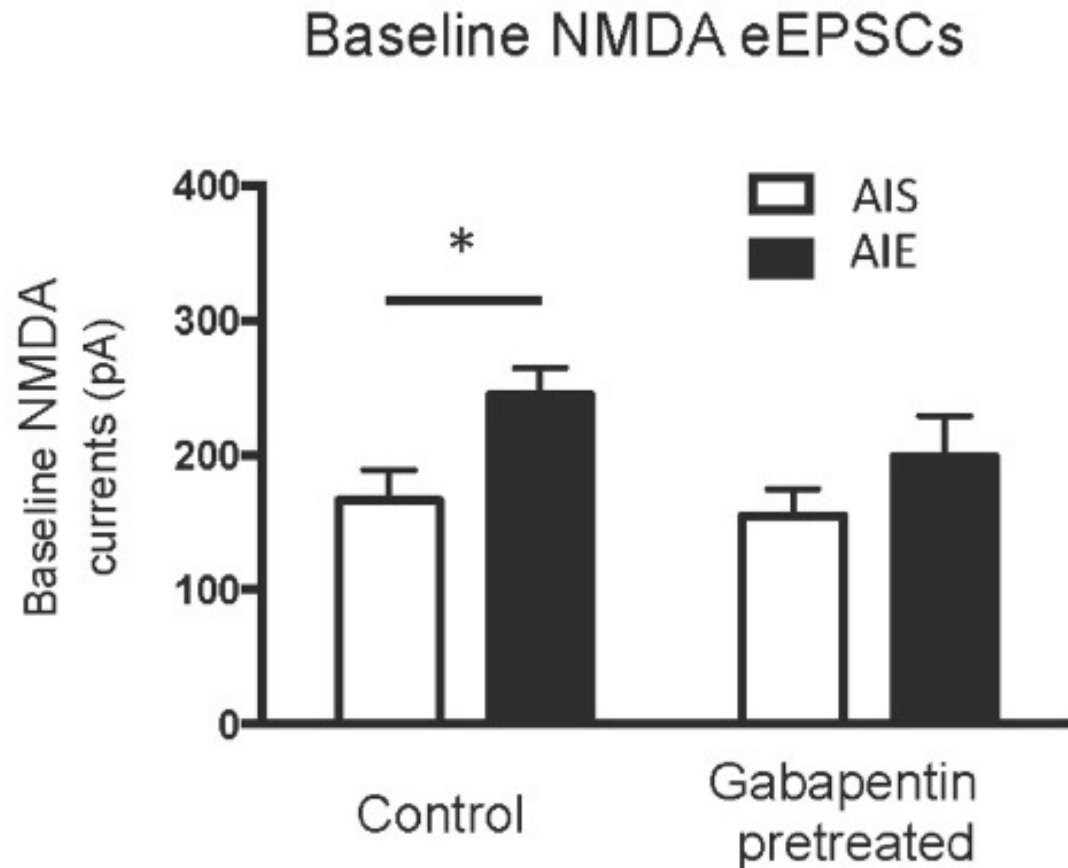
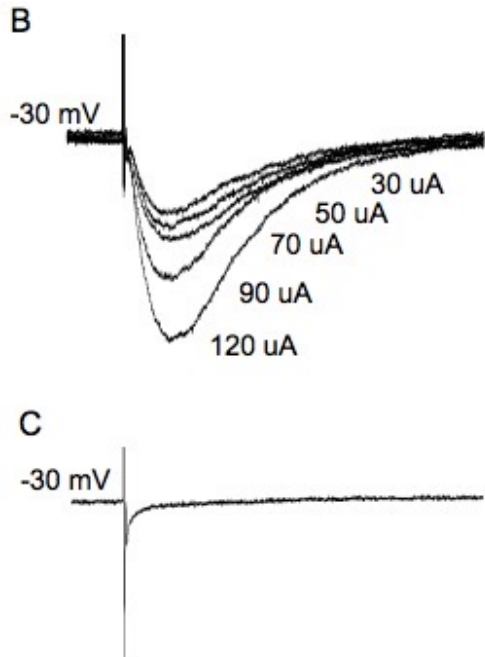


# Possible Mechanism: Reduction of cholinergic input to hippocampal formation



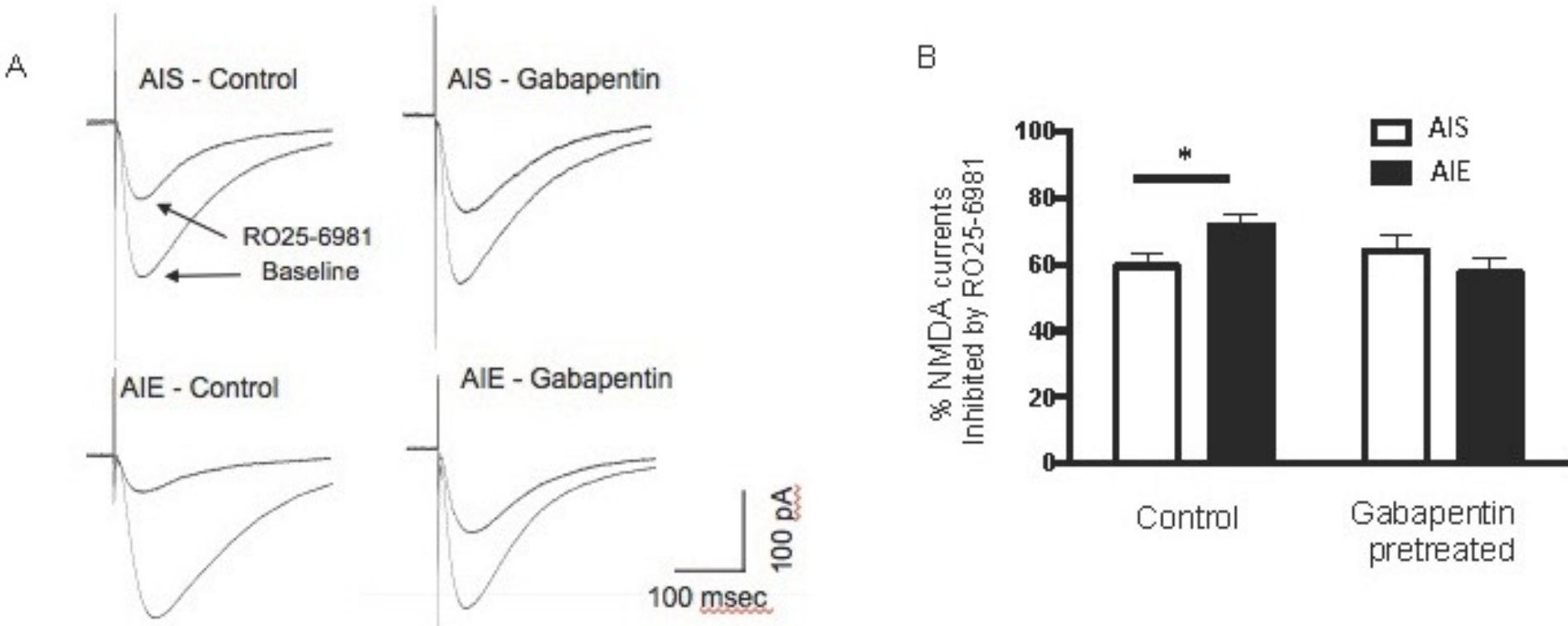


# Gabapentin attenuates AIE-induced increases in NMDAR-mediated EPSCs in CA1 pyramidal cells

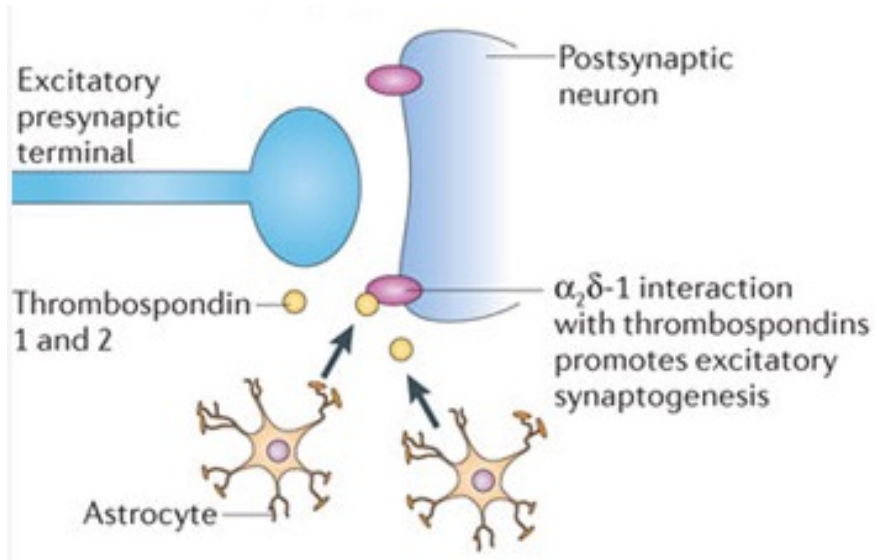


# Gabapentin also reverses AIE-induced increase in GluN2B drive of NMDA currents

## Shift Toward GluN2B Current Drive



# Possible Mechanism: Increase of Astrocyte-mediated excitatory synaptogenesis



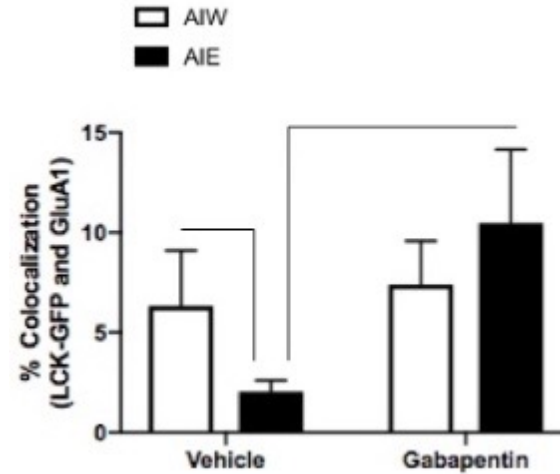
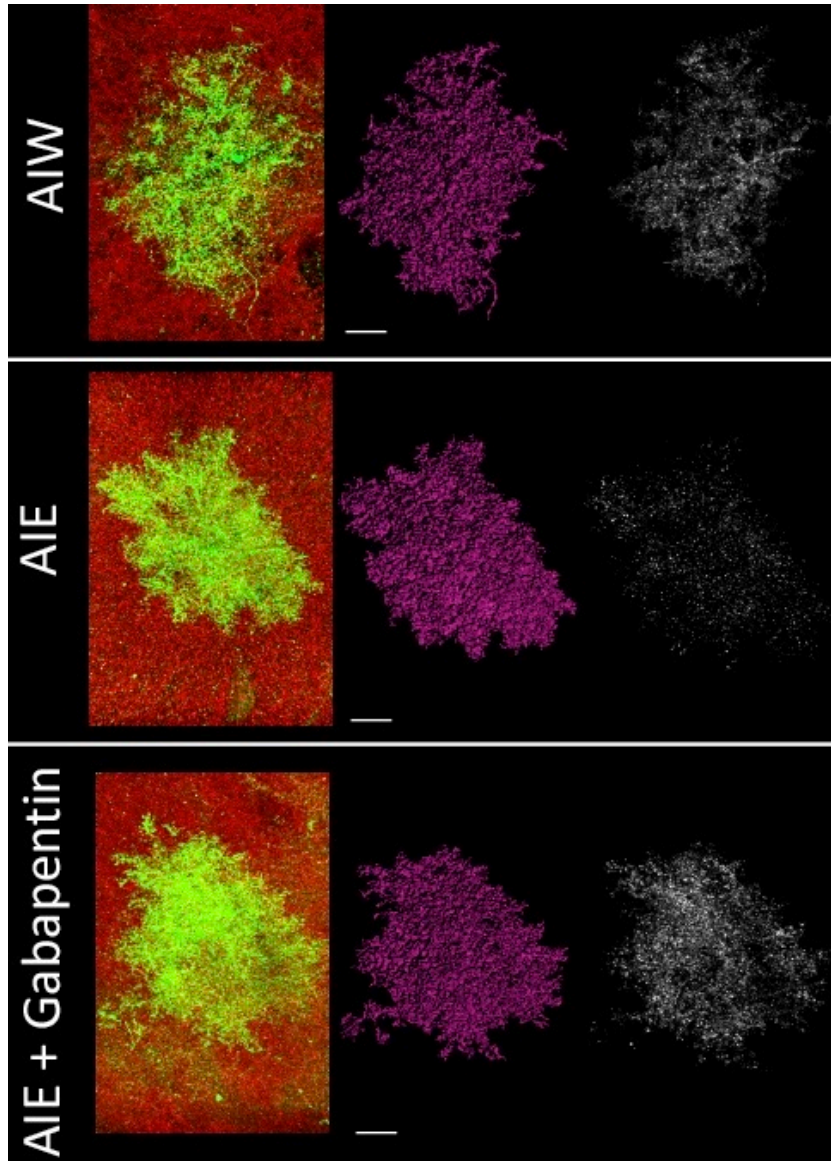
Astrocytes regulate neuronal synaptogenesis and function

We reported upregulation of thrombospondins and the  $\alpha_2\delta-1$  receptor in CA1 after AIE.

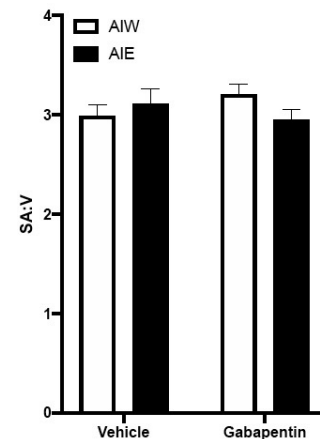
Upregulated TSP's increase excitatory synaptogenesis → hyperexcitability after AIE (and spine changes).

Gabapentin antagonism of the  $\alpha_2\delta-1$  receptor could reverse that process.

# AIE reduces astrocyte-synaptic proximity in HPC – reversal by gabapentin



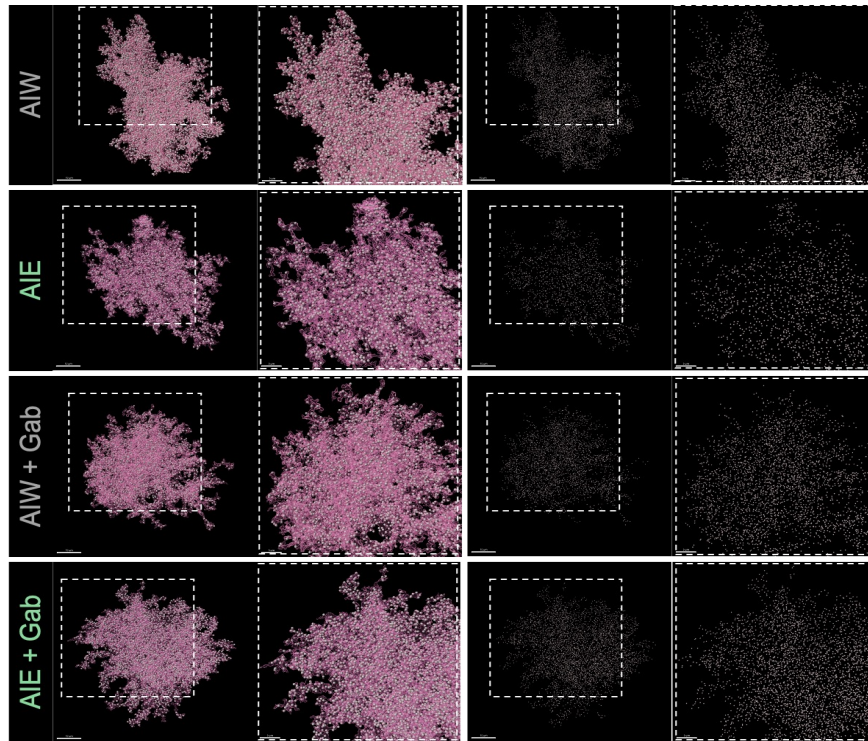
AIE induces a reversible decrease of astrocyte-synaptic proximity in CA1



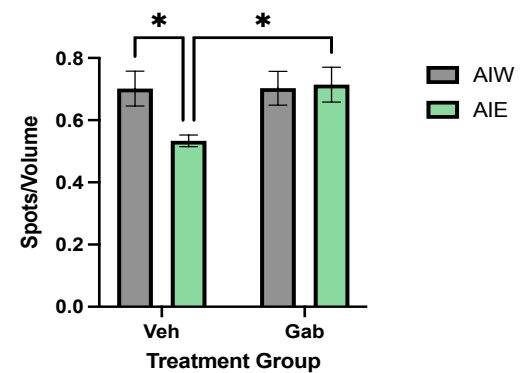
No effect on astrocyte gross morphology



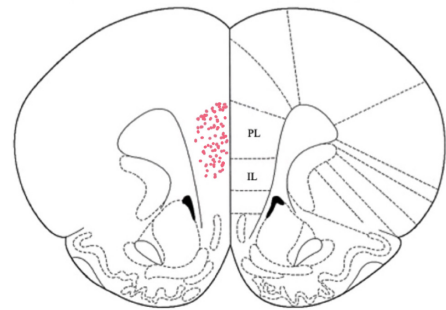
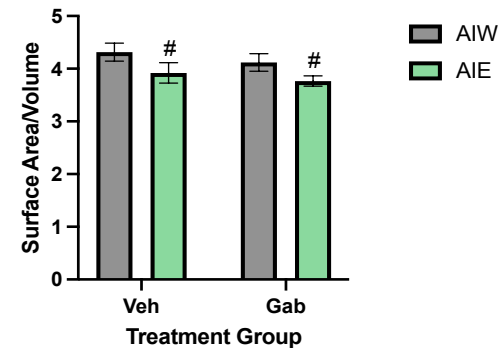
# AIE reduces astrocyte-synaptic proximity in mPFC – reversal by gabapentin



ROI Colocalization Density



Astrocyte Morphology



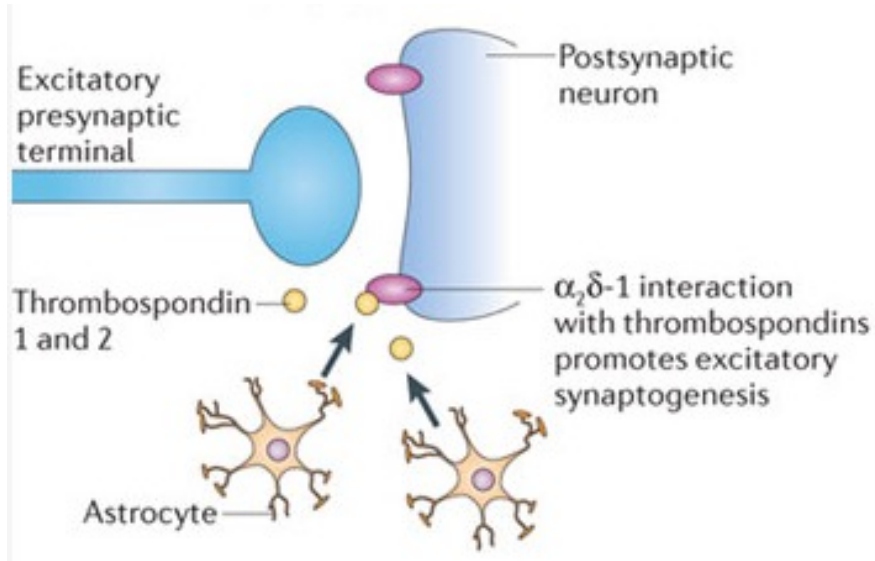
# **Adolescent Brain and Alcohol**

- **More sensitive to memory impairment**
- **Less sensitive to sedative effects**
- **Easier to drink to brain impairment without realizing it.**
- **Repeated exposure (AIE) alters HPC structure and function in adulthood**
- **Some adolescent phenotypes appear ‘locked-in’**
- **Neuroinflammatory, epigenetic, and astrocytic mechanisms**
- **Clinical drugs that reverse AIE effects**



# Lock-In

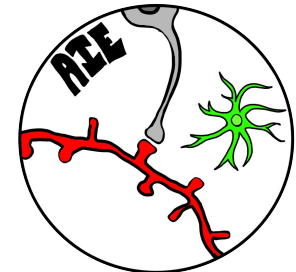
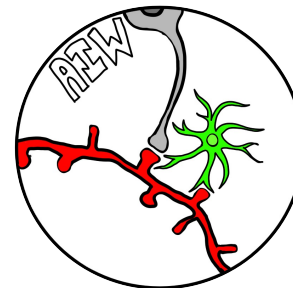
## Adolescent-Typical Astrocyte-Neuronal Interaction



Astrocytes regulate neuronal synaptogenesis and function

AIE upregulates thrombospondins and the  $\alpha_2\delta-1$  receptor in HPC, and causes retraction of astrocytes from the synaptic region.

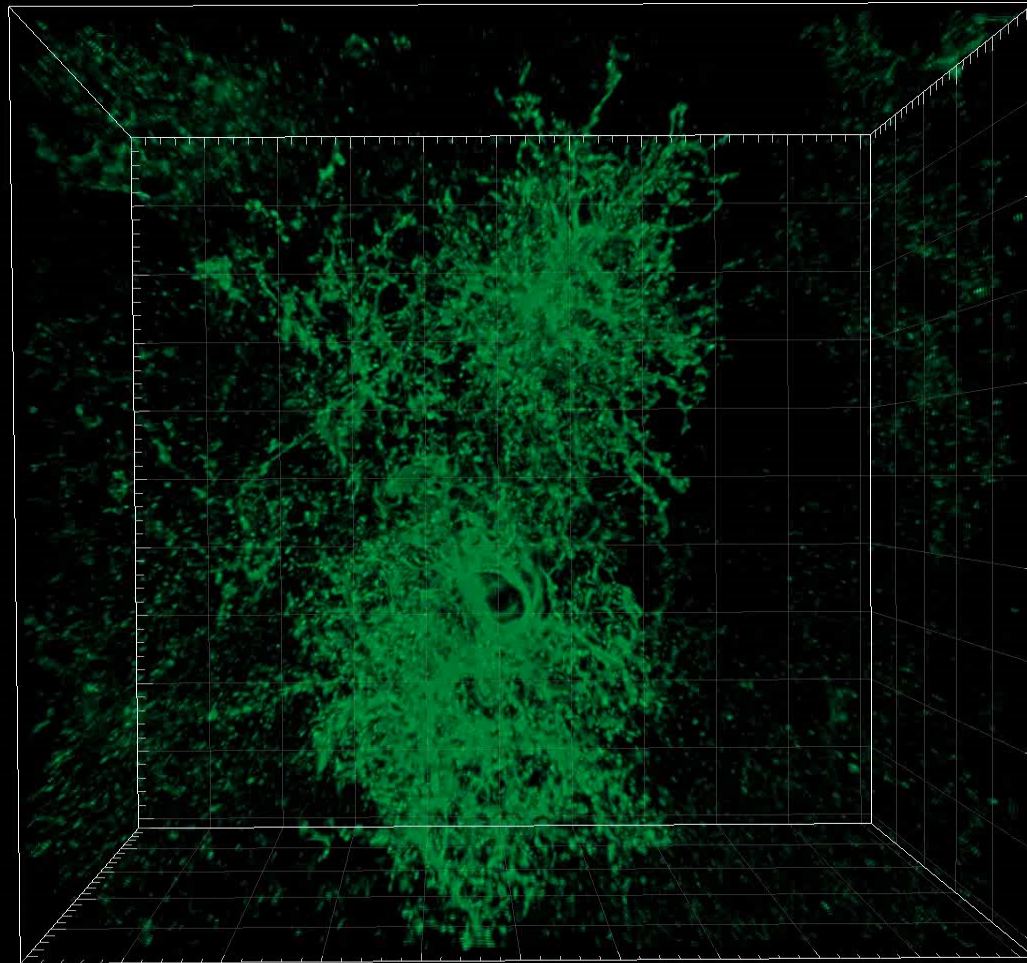
Disrupting synaptic regulation





# Lock-In

## Adolescent-Typical Astrocyte-Neuronal Proximity



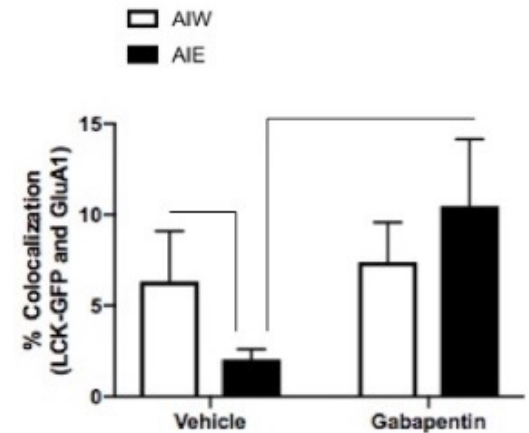
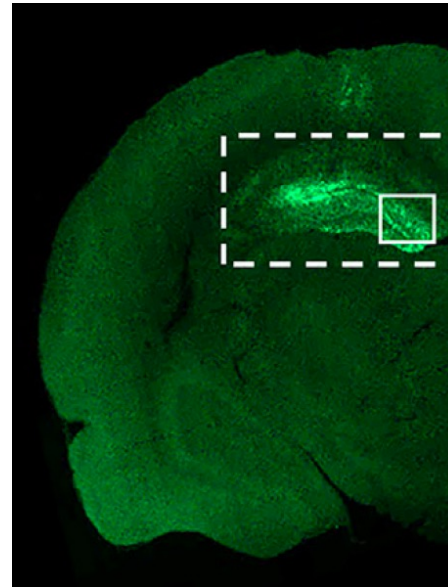
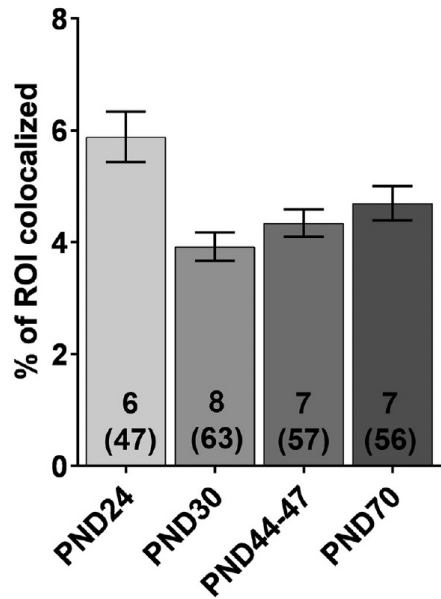
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# Lock-In

## Adolescent-Typical Astrocyte-Neuronal Proximity: dHPC

Proximity lower during adolescence

Proximity lower in adulthood after adolescent alcohol exposure

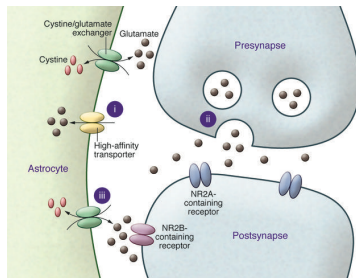
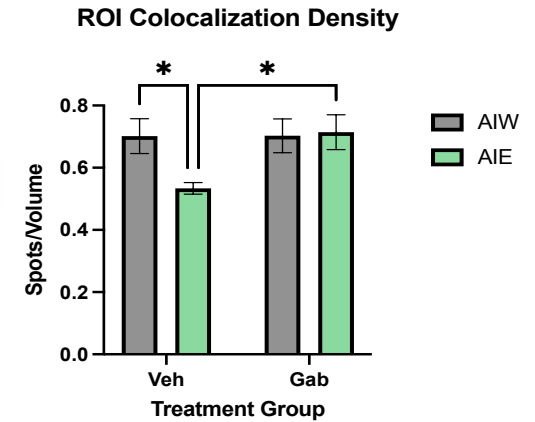
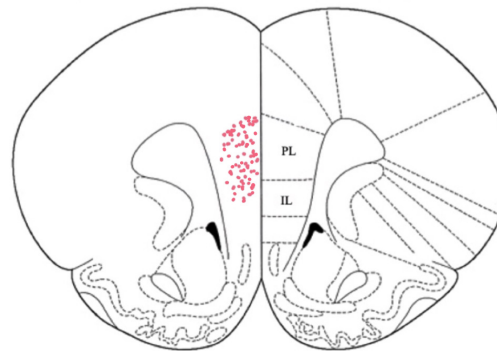
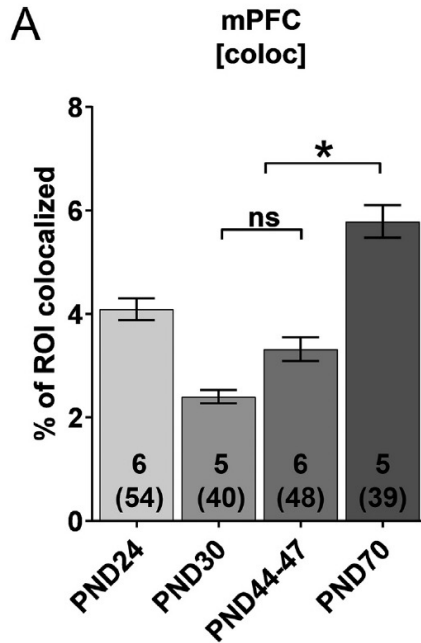


# Lock-In

## Adolescent-Typical Astrocyte-Neuronal Proximity: mPFC

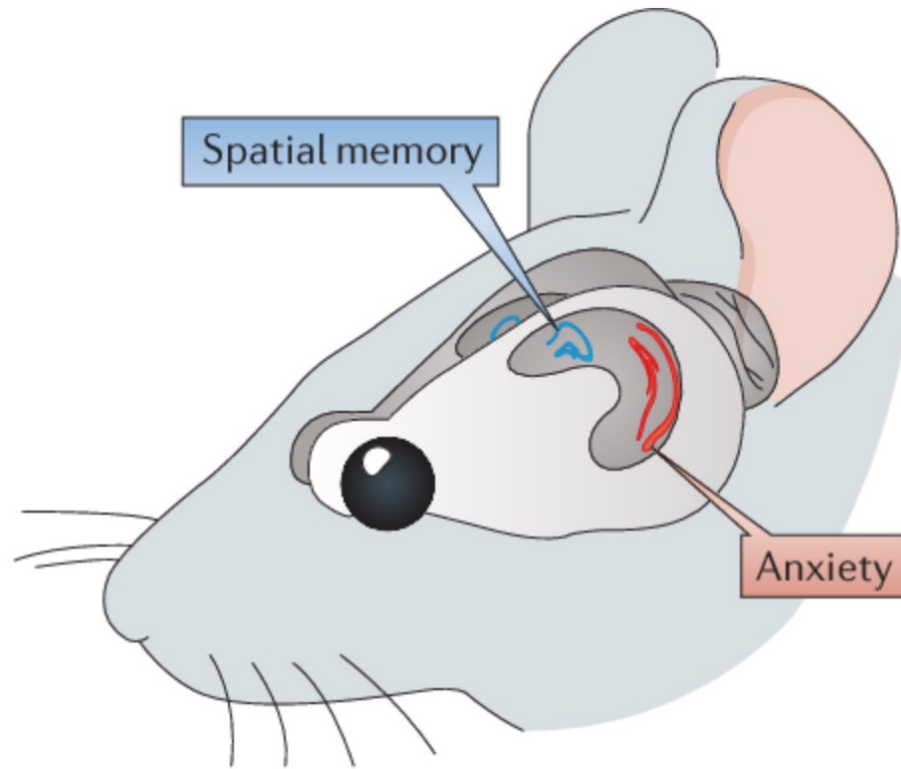
Proximity lower during adolescence

Proximity lower in adulthood after adolescent alcohol exposure

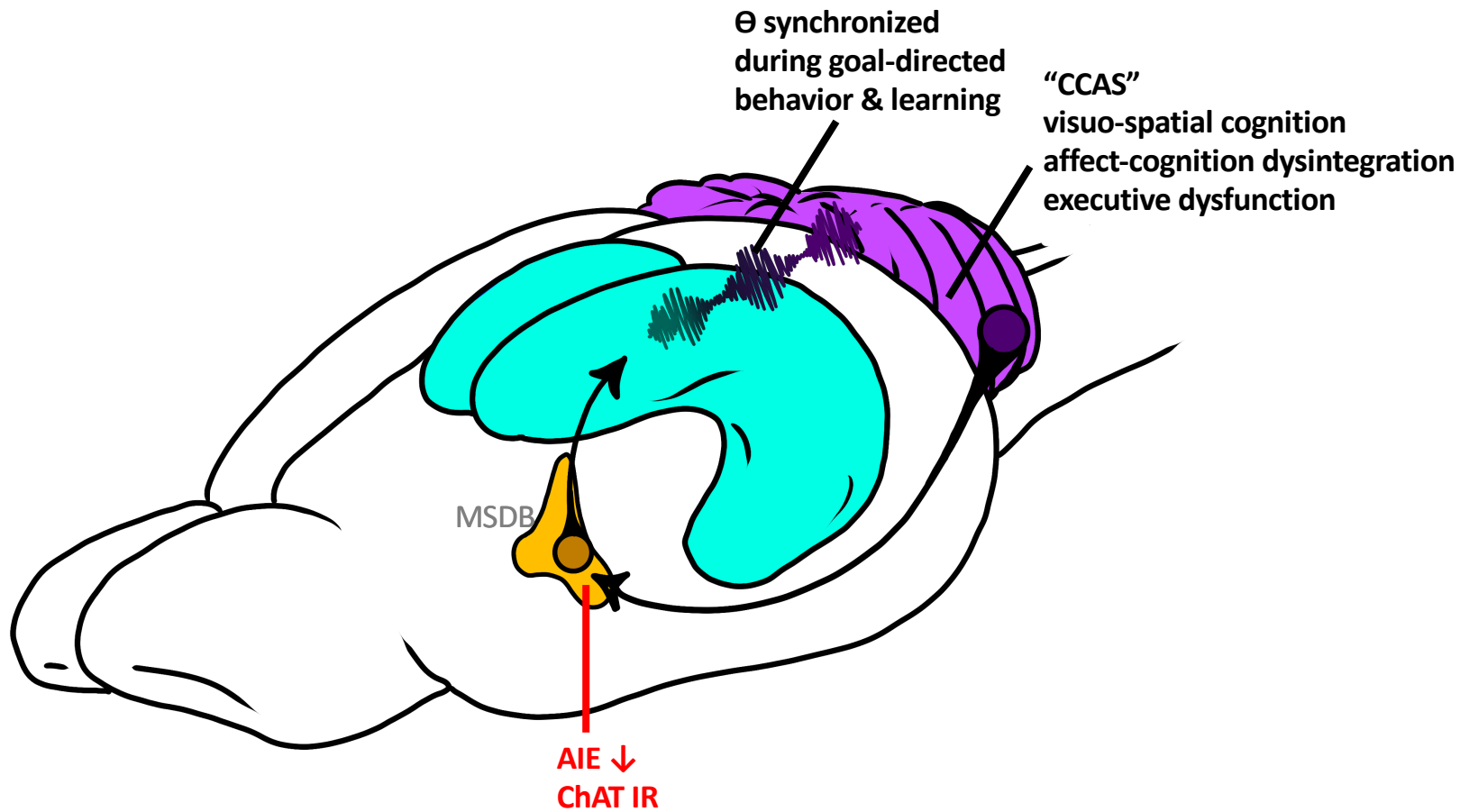


Diminished efficiency of the 'tripartite synapse' in both HPC and mPFC should impact cognition, affect, their integration, and self-regulation.

# HPC subregions interactively drive memory and affective behavior



Each subregion engages extra-hippocampal circuits

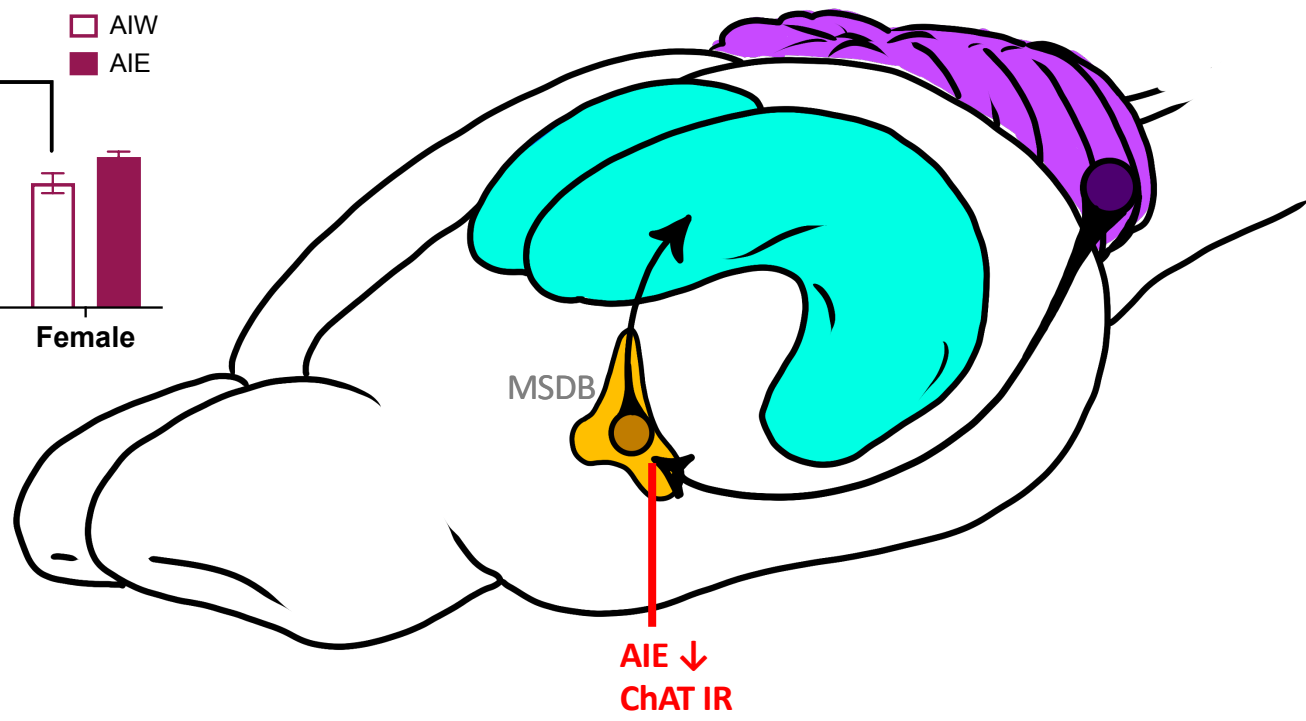
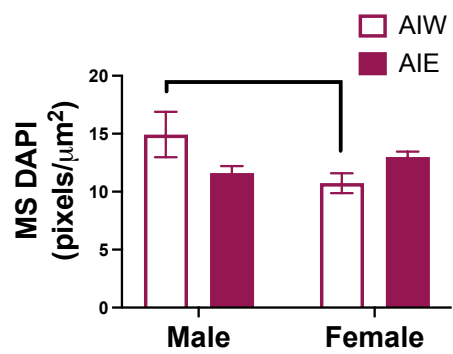
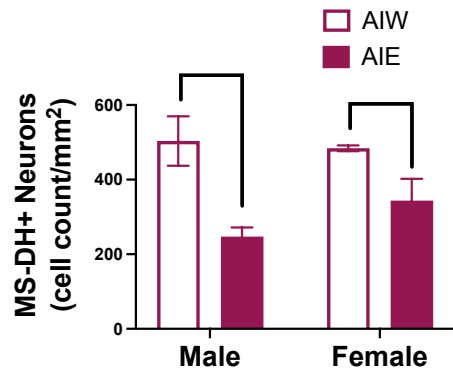


## Extra-Hippocampal Circuits of Interest

### Cerebellum



Retrobeads infused into dHPC and counted in medial septal somata

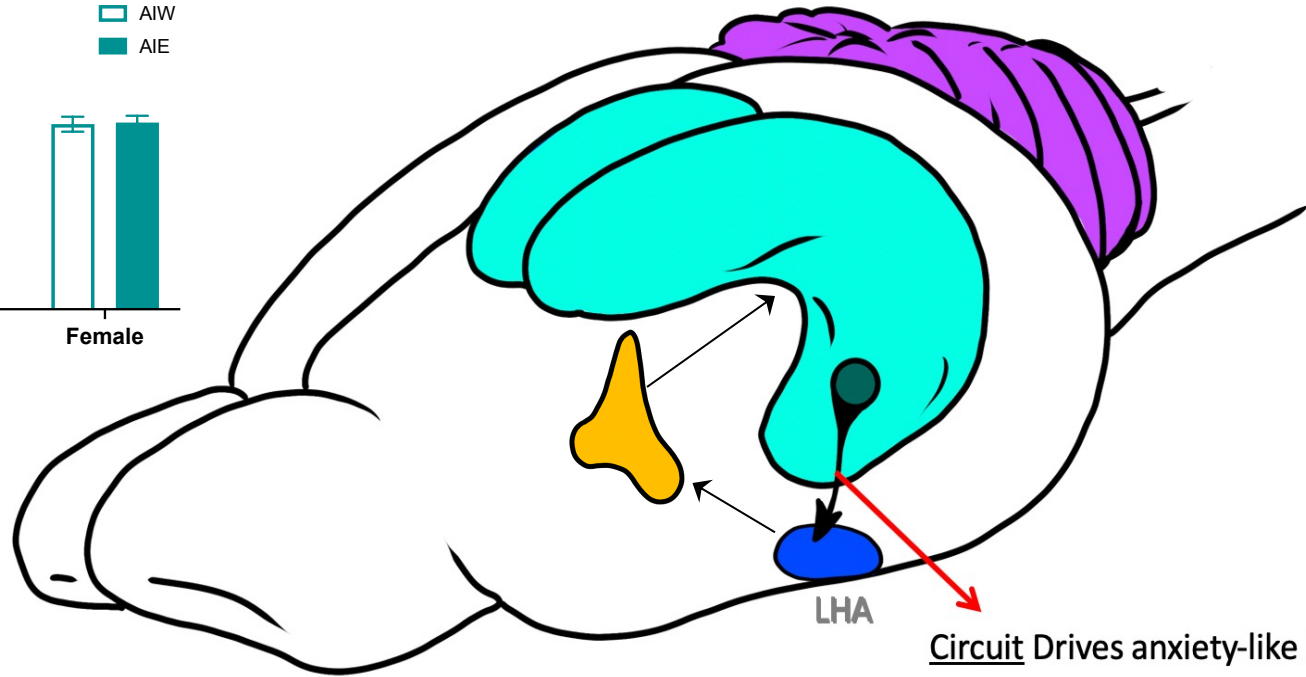
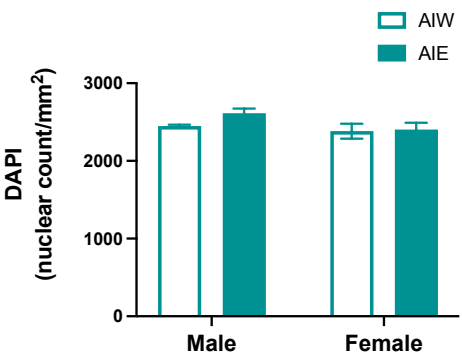
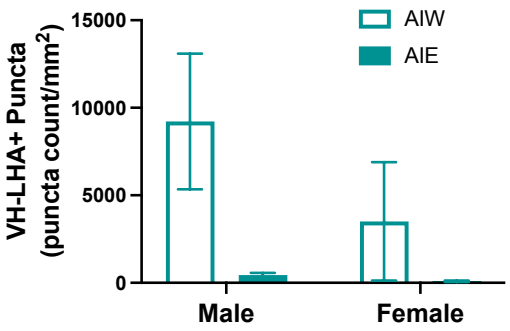


Extra-Hippocampal Circuits of Interest

MSDB -> dHPC Projection



Retrobeads infused into LHA. Bead puncta counted in vHPC



Circuit Drives anxiety-like behavior

LHA modulates inflammation, pain transmission, reward processing  
Projects to MSDB influencing risk assessment

## Extra-Hippocampal Circuits of Interest

vHPC -> LHA Projection