

[EXHIBIT F2]

New Research Techniques in the Field of Alzheimer's Disease

Steve Barger, Ph.D.

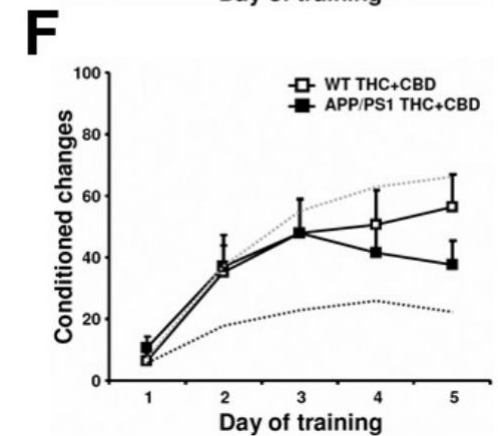
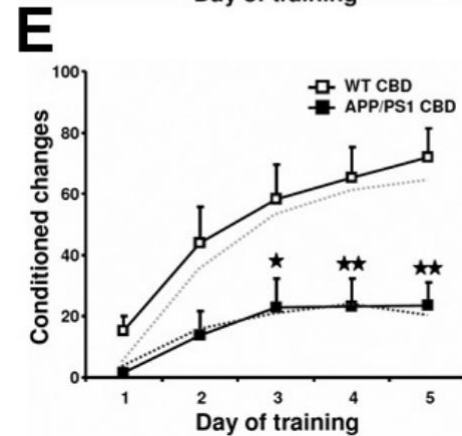
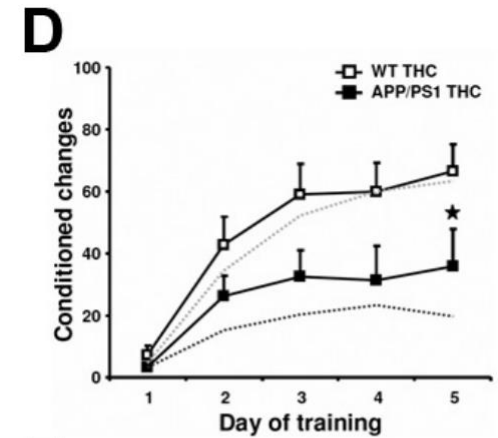
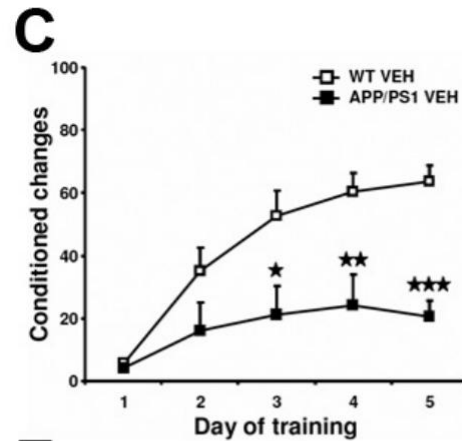
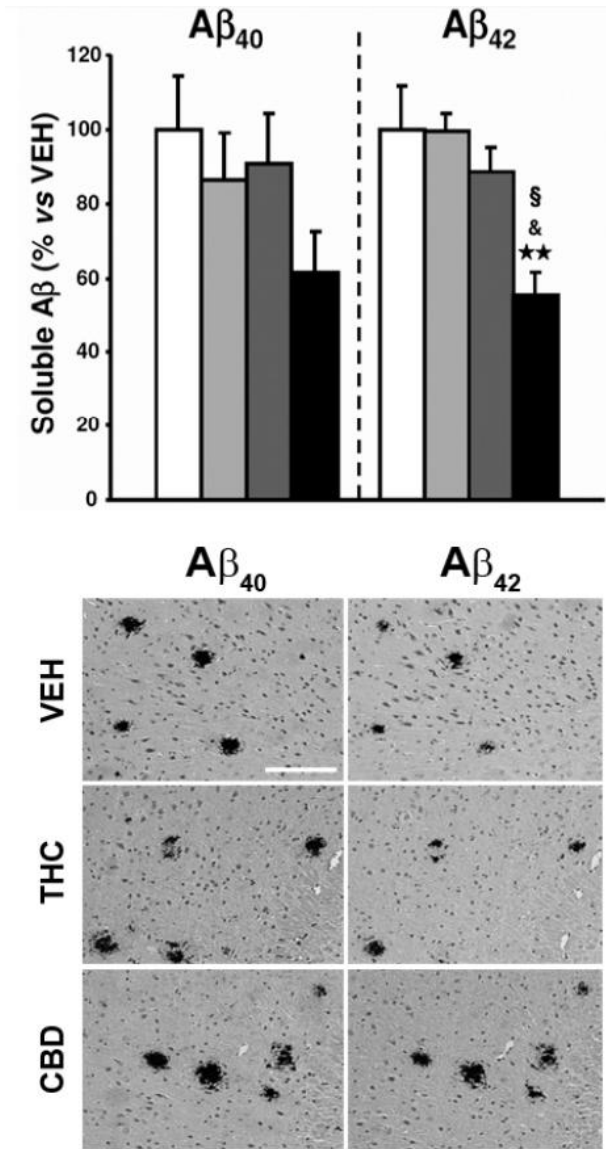
December 12, 2016

Correlation is not causality

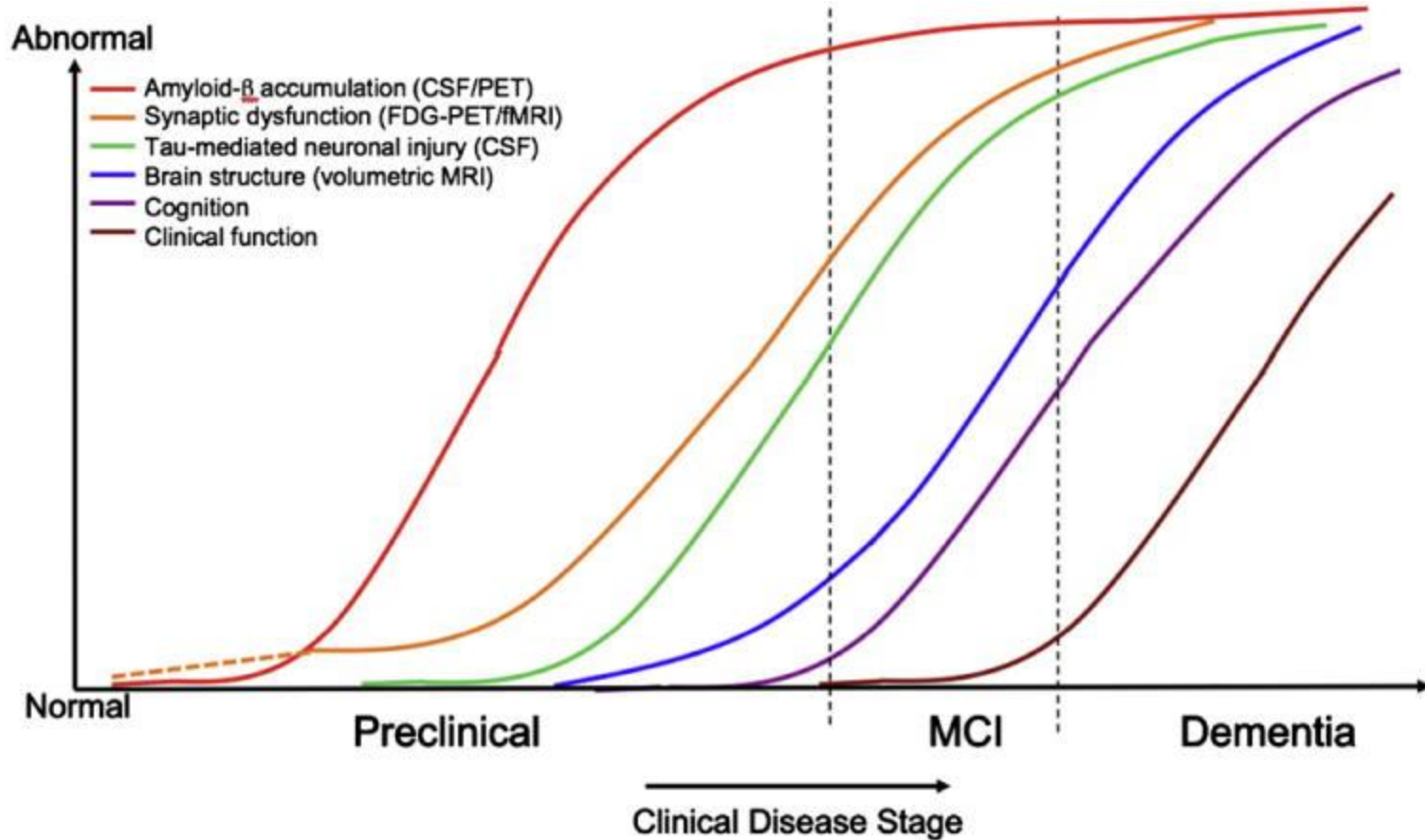
Spurious associations:

EATING OR DRINKING	IS LINKED TO	P-VALUE
Raw tomatoes	Judaism	<0.0001
Egg rolls	Dog ownership	<0.0001
Energy drinks	Smoking	<0.0001
Potato chips	Higher score on SAT math vs. verbal	0.0001
Soda	Weird rash in the past year	0.0002
Shellfish	Right-handedness	0.0002
Lemonade	Belief that “Crash” deserved to win best picture	0.0004
Fried/breaded fish	Democratic Party affiliation	0.0007
Beer	Frequent smoking	0.0013
Coffee	Cat ownership	0.0016
Steak with fat trimmed	Lack of belief in a god	0.0030
Iced tea	Belief that “Crash” didn’t deserve to win best picture	0.0043
Bananas	Higher score on SAT verbal vs. math	0.0073
Cabbage	Innie bellybutton	0.0097

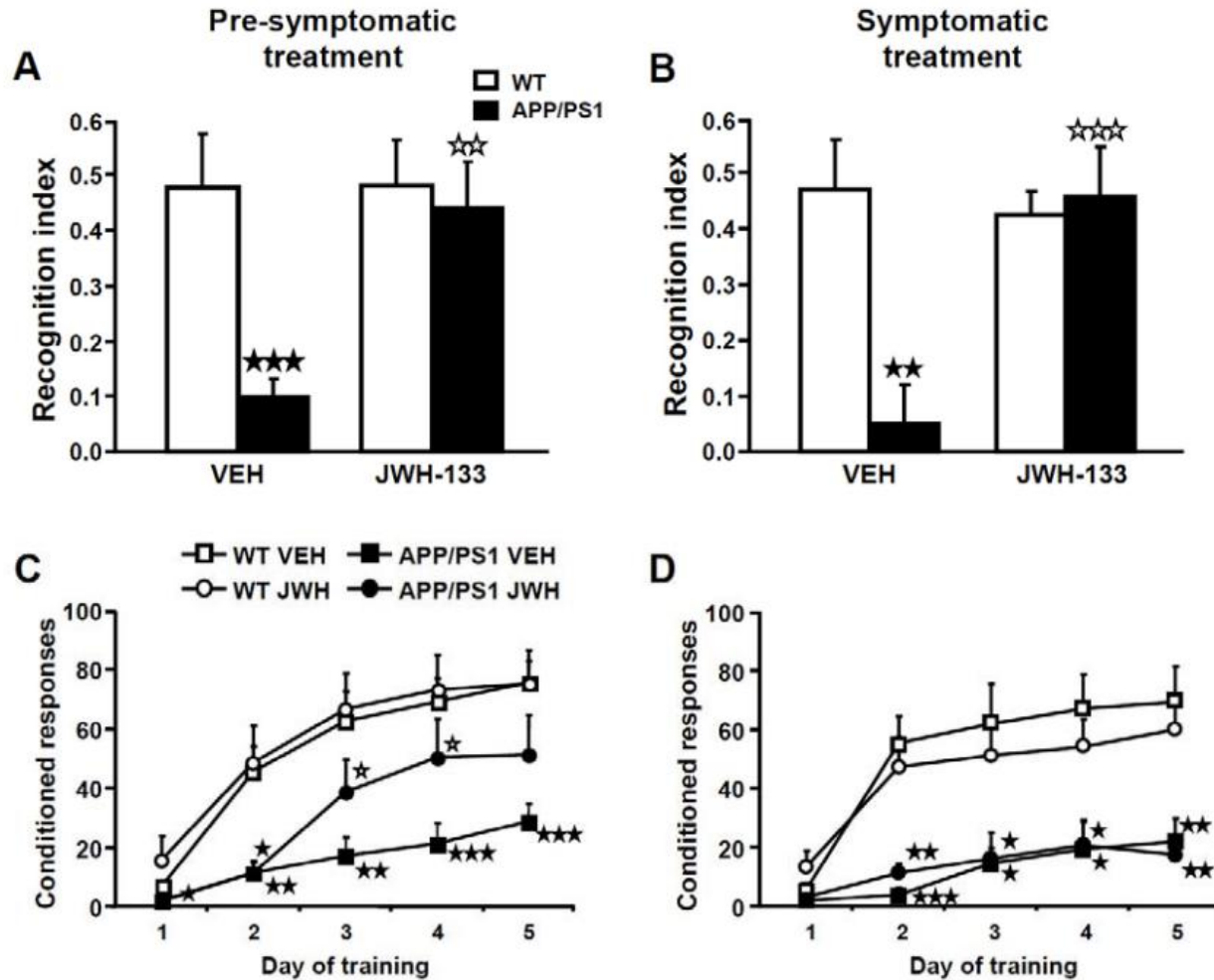
Cannabinoid-induced improvements in a mouse model of Alzheimer's disease pathology



Chronology of AD biomarkers suggests the order of events (and many years of disease development)



Need for new techniques

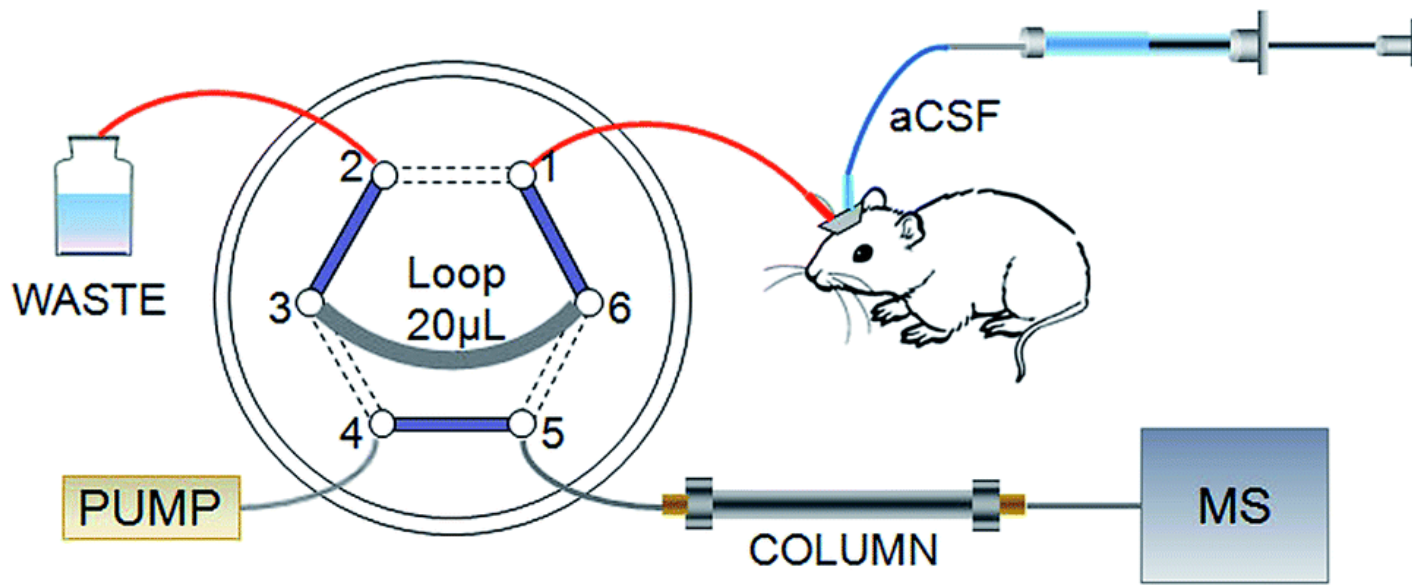


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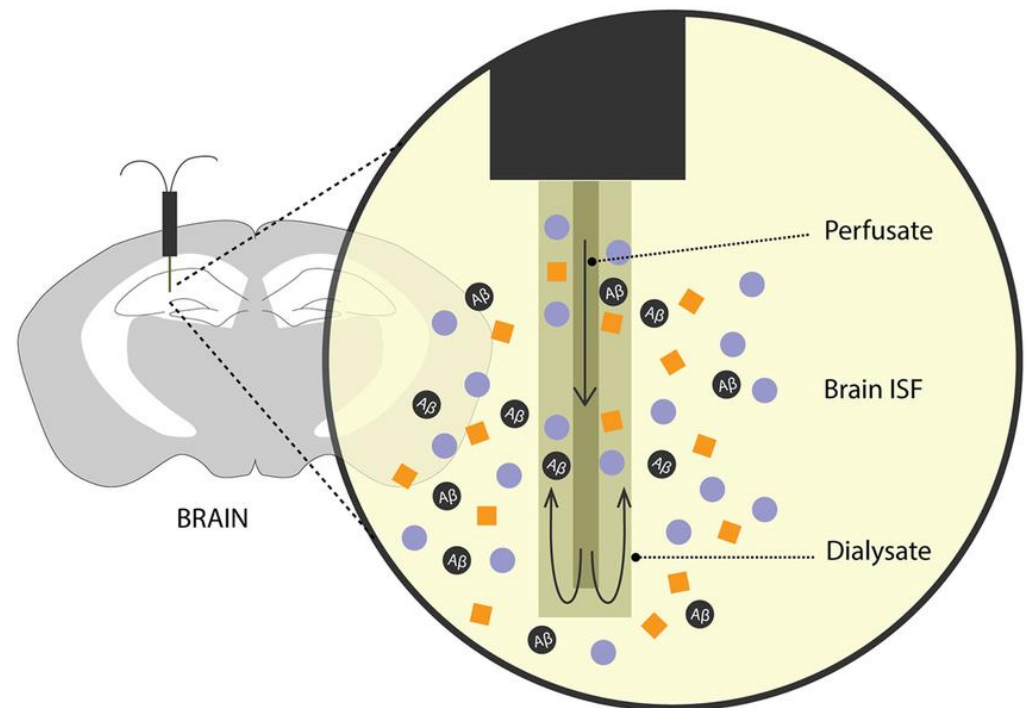
- Passive:
Sensing / detection / assaying
- Active:
Manipulating neural systems

Passive (detection)

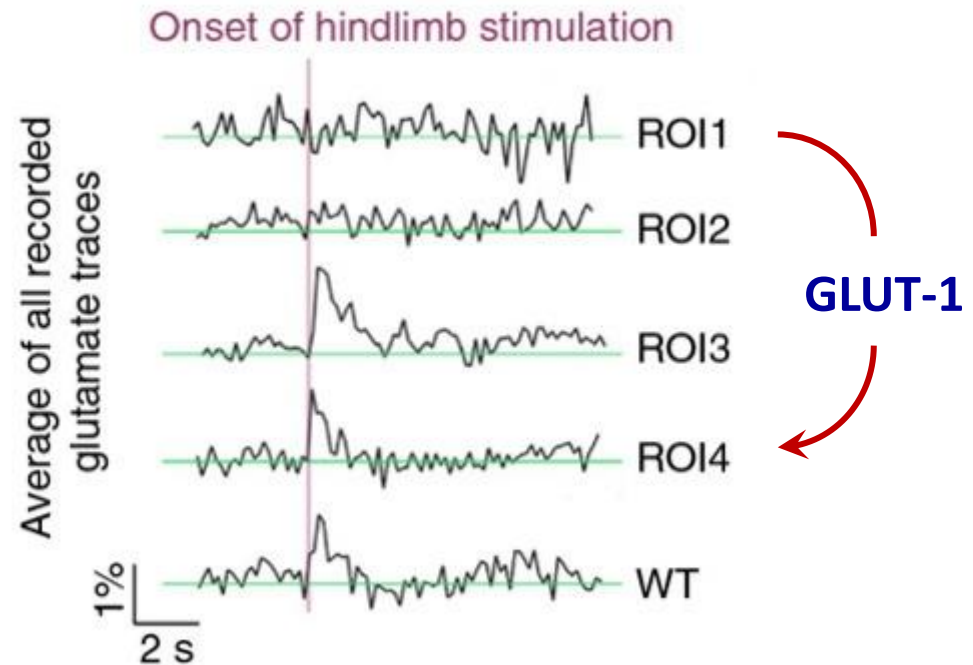
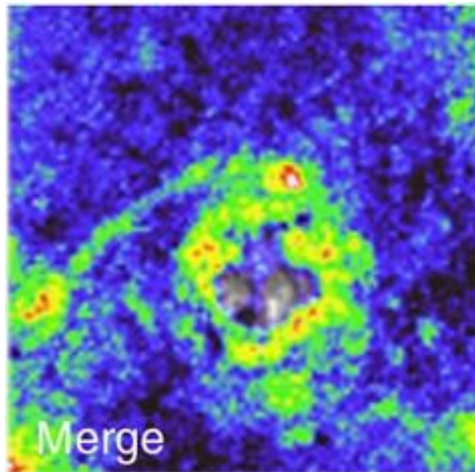
- **Microdialysis:**
Taking liquid samples to assay molecules – such as neurotransmitters – released into the spaces between brain cells
- **Imaging:**
Non-invasive visualization of molecular probes that can be introduced by genetics or by injection



Microdialysis of mouse brain



Imaging: Probe for glutamate (a neurotransmitter)

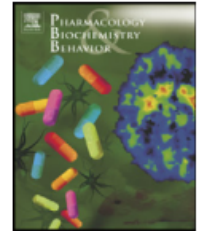




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Role of GLT-1 transporter activation in prevention of **cannabinoid** tolerance by the beta-lactam antibiotic, ceftriaxone, in mice

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ABSTRACT

Recently, it has been indicated that beta lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Furthermore, these antibiotics have been shown to prevent the development of tolerance and dependence to opioids. Since cannabinoid tolerance is known to be similar to opioids, our purpose was to examine the effect of ceftriaxone on the development of tolerance to WIN 55,212-2, a cannabinoid agonist. The tail flick test, a rectal thermometer, and the ring test were used for evaluating the degree of tolerance to the analgesic, hypothermic, and cataleptic effects of WIN 55,212-2, respectively. Within one week, animals became completely tolerant to analgesic, hypothermic and cataleptic effects of WIN 55,212-2 (6 mg/kg). Ceftriaxone, with its higher doses (100–200 mg/kg), attenuated the development of tolerance to the analgesic and hypothermic effects of WIN 55,212-2, but had no effect on its cataleptic action. Dihydrokainic acid (10 mg/kg), a GLT-1 transporter inhibitor, prevented this effect of ceftriaxone. Our results suggest that repeated treatment with ceftriaxone prevents the development of tolerance to the analgesic and hypothermic effects of cannabinoids, and GLT-1 activation appears to play a key role in this preventive effect of beta-lactam antibiotics.

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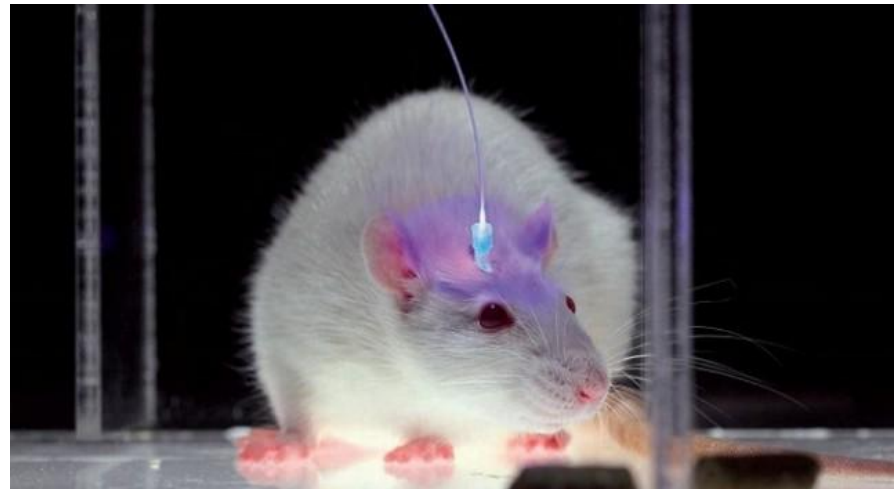
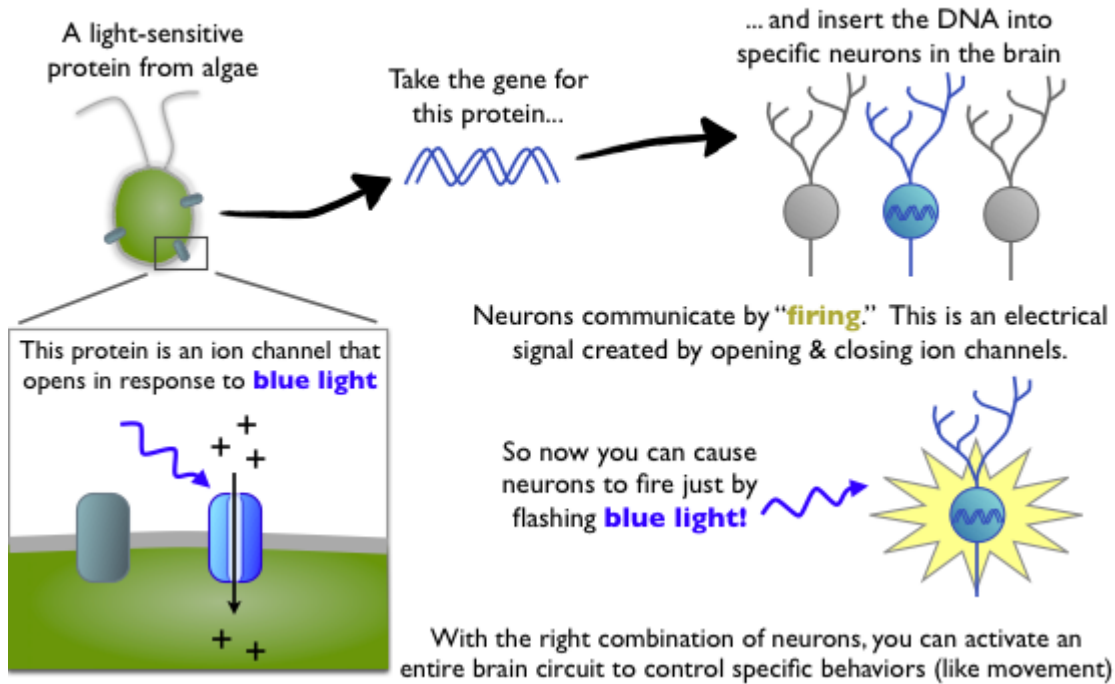
Active (manipulation)

- Optogenetics
- DREADDS

Optogenetics

- Uses light to activate brain cells
- Very fast “on” and “off”
- Often “invasive”
(requires fiber optics to reach deep brain regions)
- Can be targeted at a specific cell or a class of cells

How optogenetics works



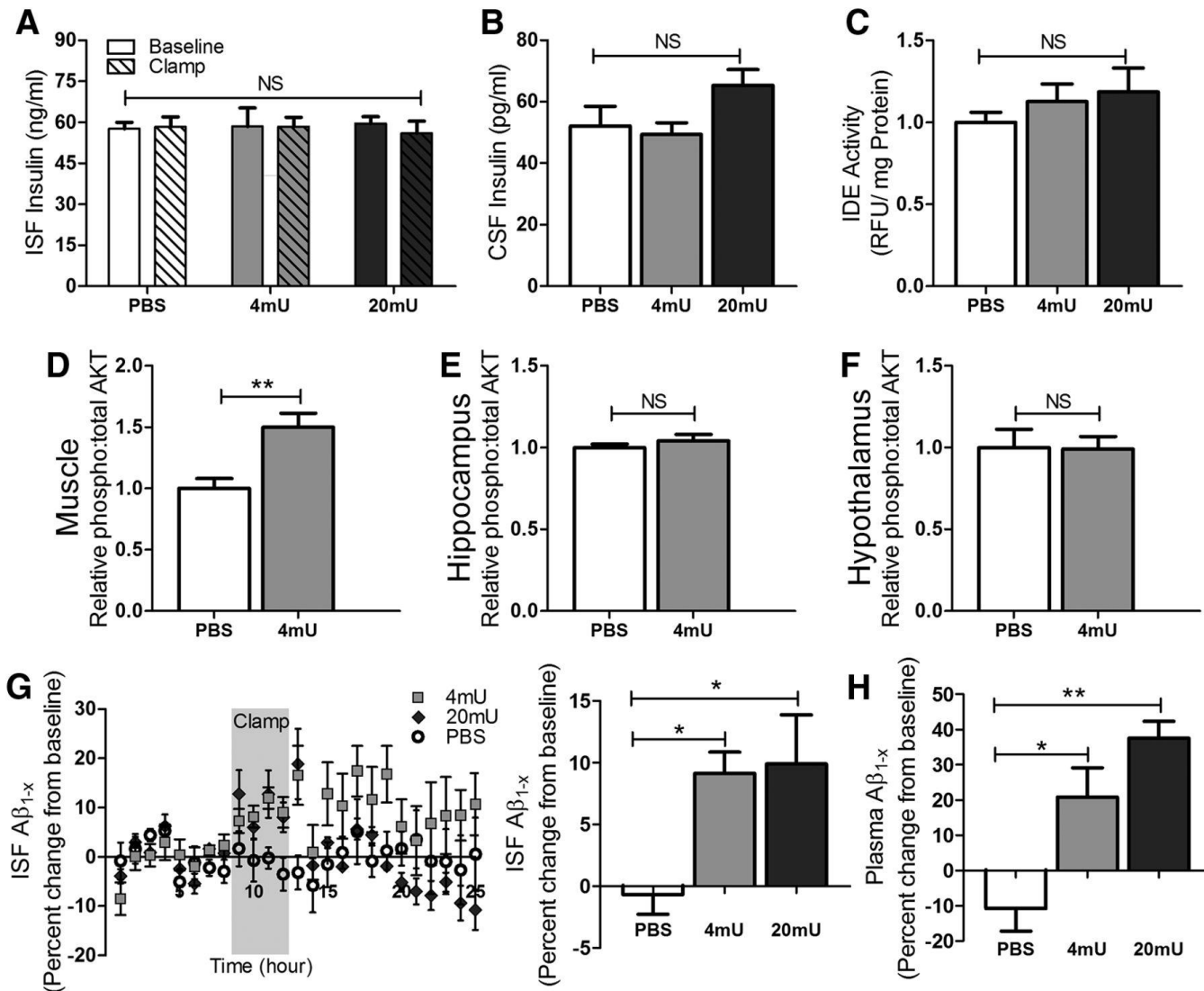
DREADDs

“Designer Receptors Exclusively Activated by Designer Drugs”

“Designer receptor”: a protein genetically engineered to activate a cellular function but not itself activated by normal bodily hormones

“Designer drug”: a man-made molecule with a shape and other chemical properties engineered to bind to another molecule (the latter being a designer receptor, in this case)

Active *and* Passive: Monitoring while manipulating



Active *and* Passive: Monitoring while manipulating

