Electrophysiology

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Alcohol Research Center Virtual Internship Program

Scripps postdoctoral assistant/outside resource specialist

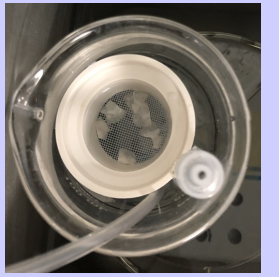
Picture credit: Alicia Avelar, PhD

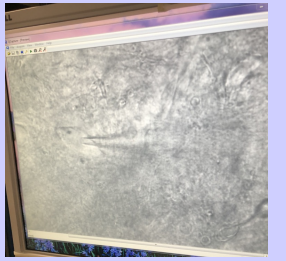
UT Health San Antonio

Professional pic

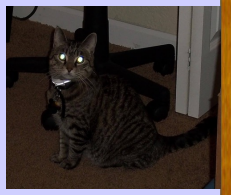
Scripps Research

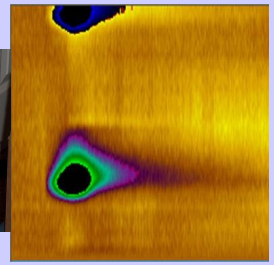
TRITONS UC San Diego





Illinois State Redbirds





Marshall

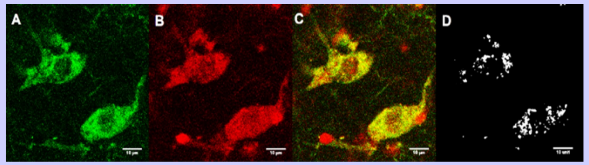
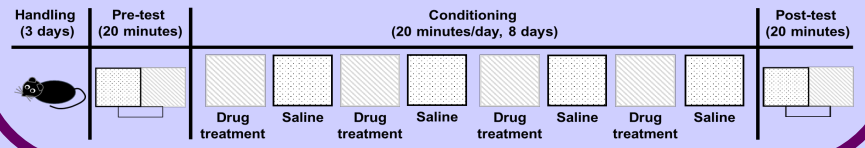


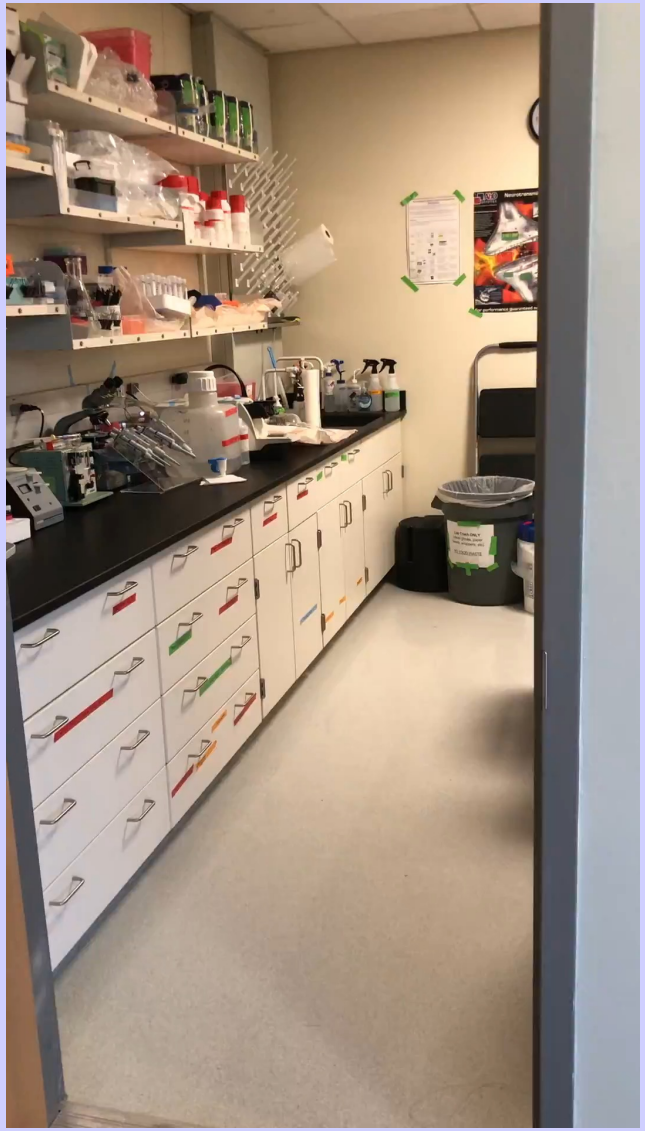
Figure 5. Confocal images of midbrain dopamine neurons in mouse brain slices. A. Dopamine neurons containing nACh receptor α6-GFP subunits. B. Dopamine neurons containing nACh receptor α4-mCherry subunits. C. Images A and B merged. D. Normalized Förster Resonance Energy Transfer (NFRET) images, showing locations where both α6-GFP and α4-mCherry nACh receptor subunits are present.



University of San Diego Est. 1949

Non logo pictures, data, and CPP image credit: Alicia Avelar, PhD

Electrophysiology lab tour



George lab at University of California San Diego, 2021

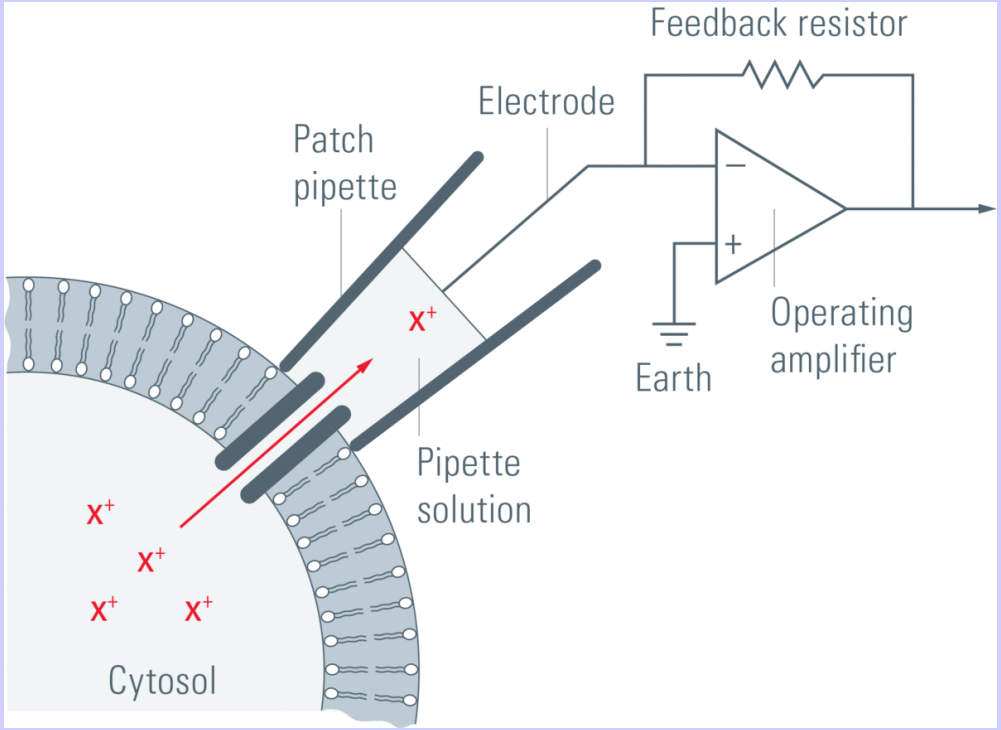
Currents from ions flowing through channels can be measured using electrophysiology (Ephys)

Table 2.1 Intracellular and extracellular distribution of the main ions found in animal fluids

Ion	Intracellular range (mM)	Extracellular range (mM)
Na^+	5–20	130–160
K^+	130–160	4–8
Ca^{2+}	50–1000 nM[a]	1.2–4
Mg^{2+}	10–20	1–5
Cl^-	1–60	100–140
HCO_3^-	1–3	20–30

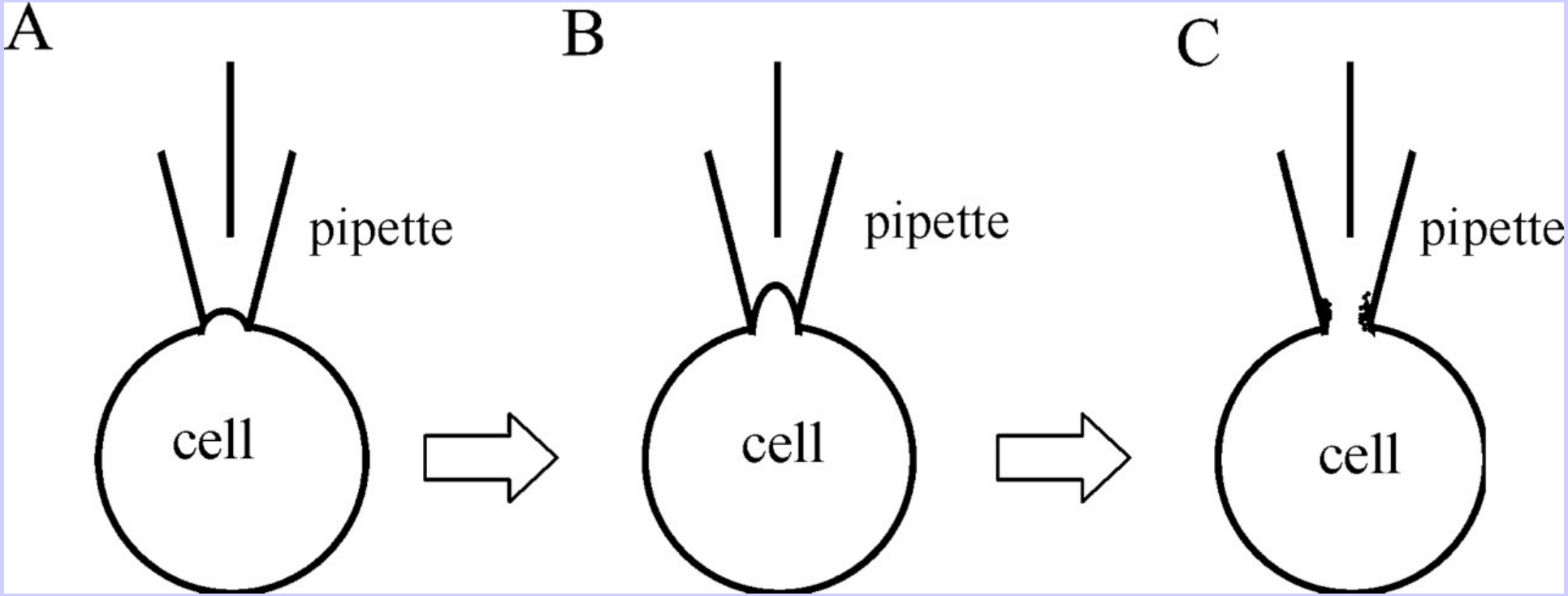
[a] Given as nanomolar rather than millimolar.

Picture of a table from an old manual. Lost citation information



https://www.leica-microsystems.com/science-lab/the-patch-clamp-technique/

Whole-cell patch-clamp electrophysiology



http://d3qpq7e7yxjovl.cloudfront.net/content/ajpadvan/32/3/209/F2.large.jpg

Postdoctoral Ephys work

Scripps Research

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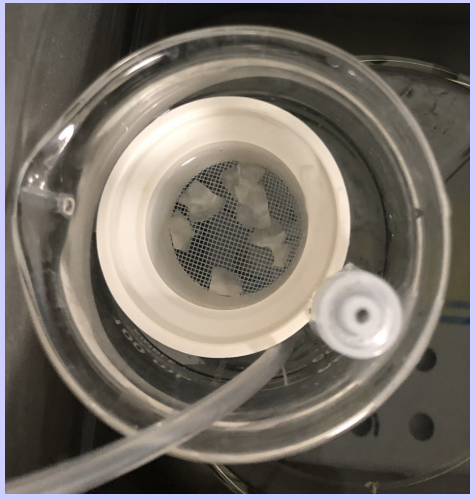
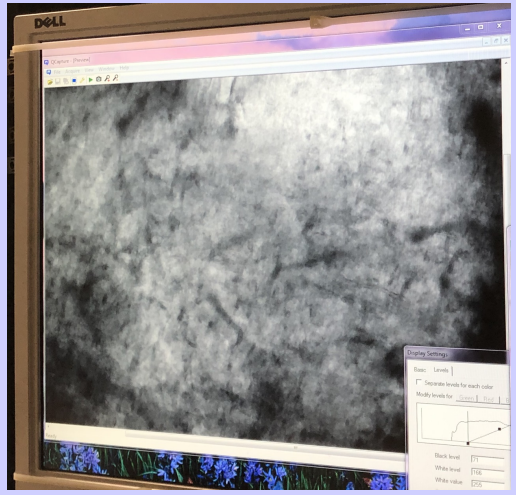
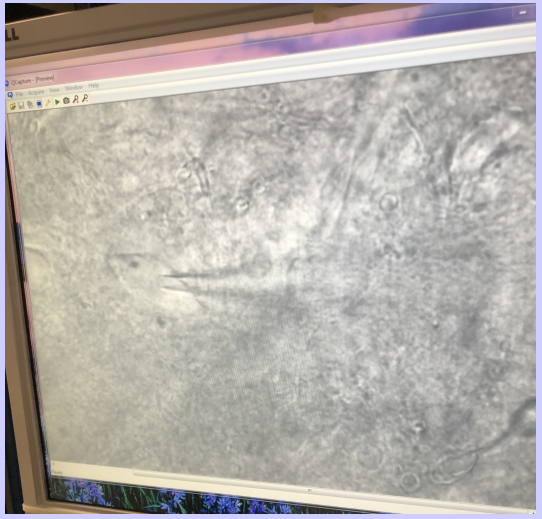
UC San Diego





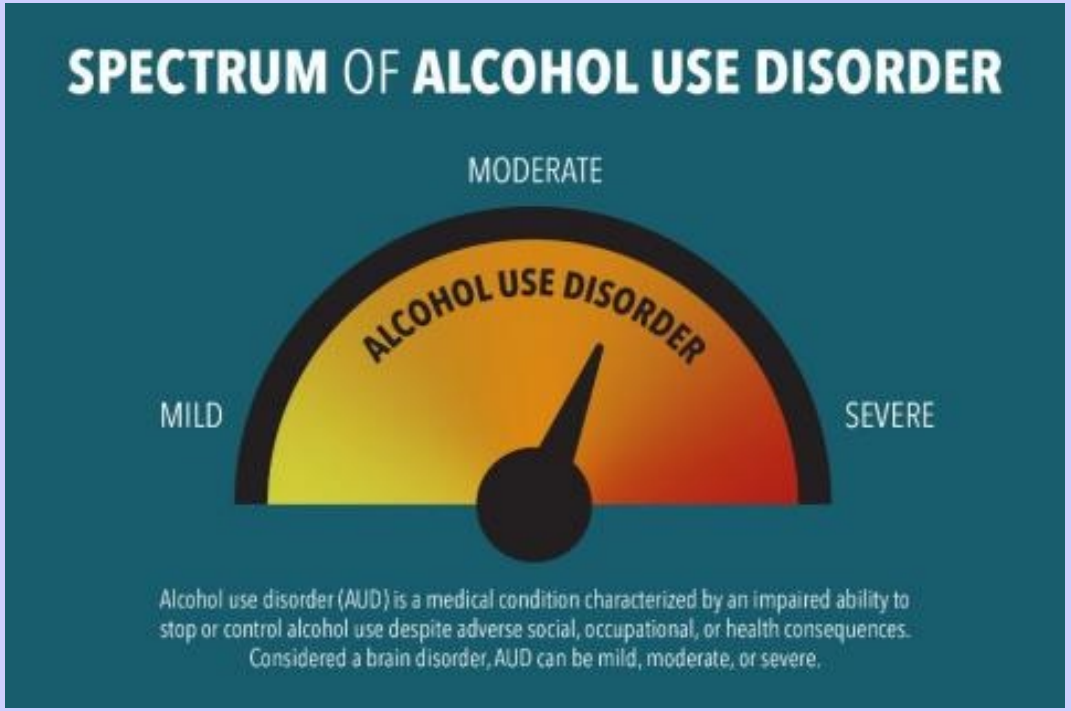


Logos from associated websites and picture credit: Alicia Avelar, PhD

Problem- Alcohol binge drinking

"Alcohol use disorder (AUD) is a medical condition characterized by an impaired ability to stop or control alcohol use despite adverse social, occupational, or health consequences."

"Prevalence of Binge Drinking and Heavy Alcohol Use: In 2019, 25.8 percent of people ages 18 or older reported that they engaged in binge drinking in the past month; 6.3 percent reported that they engaged in heavy alcohol use in the past month."
https://www.niaaa.nih.gov/publications/brochures-and-fact-sheets/alcohol-facts-and-statistics



https://www.niaaa.nih.gov/publications/brochures-and-fact-sheets/understanding-alcohol-use-disorder

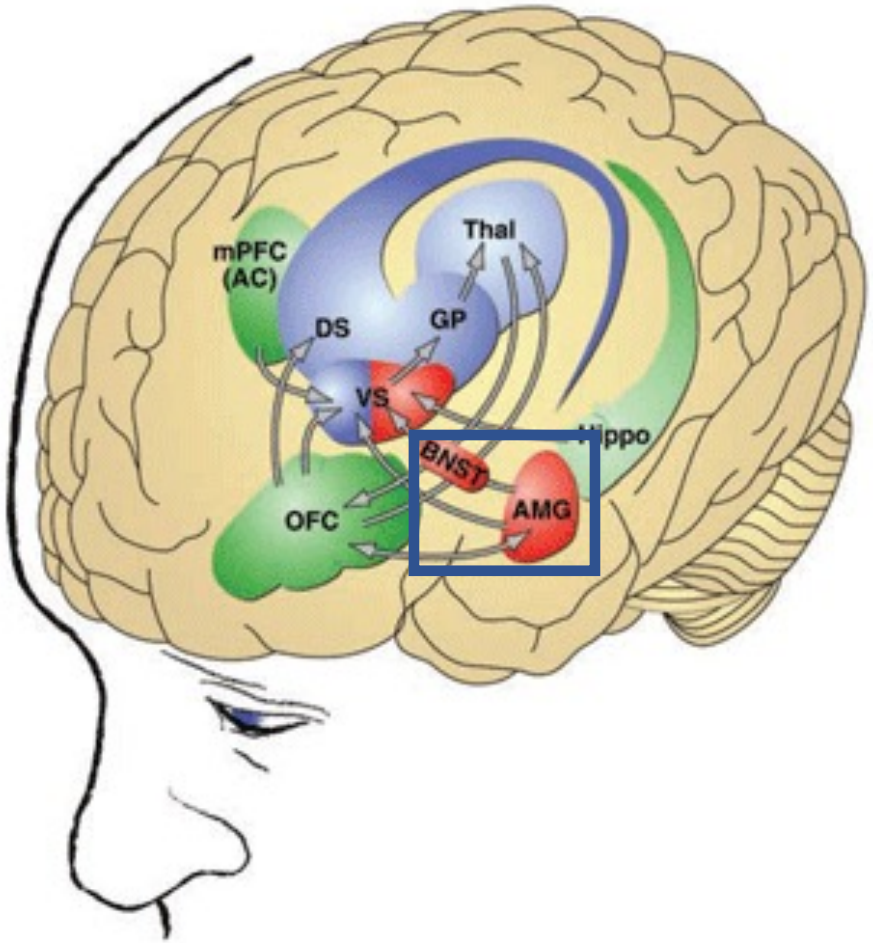
Binge drinking pattern

- “NIAAA defines binge drinking as a pattern of drinking alcohol that brings blood alcohol concentration (BAC) to 0.08 percent - or 0.08 grams of alcohol per deciliter - or higher. For a typical adult, this pattern corresponds to **consuming 5 or more drinks (male), or 4 or more drinks (female), in about 2 hours**.”

- “Emerging Trend—High-Intensity Drinking: High-intensity drinking is defined as **consuming alcohol at levels that are two or more times the gender-specific binge drinking thresholds..”**

https://www.niaaa.nih.gov/publications/brochures-and-fact-sheets/alcohol-facts-and-statistics

Amygdala involvement in ethanol use disorder



Binge/intoxication

- ventral striatum (VS), including nucleus accumbens
  euphoria, reward
- dorsal striatum (DS)
  habits, perseveration
- global pallidus (GP)
  habits, perseveration
- thalamus (Thal)
  habits, perseveration

Withdrawal/negative affect

- amygdala (AMG), bed nucleus of the stria terminalis (BNST), together also known as the "extended amygdala"
  malaise, dysphoria, negative emotional states
- ventral striatum (VS)
  decreased reward

Preoccupation/anticipation

- anterior cingulate (AC)
- prefrontal cortex (mPFC), orbitofrontal cortex (OFC)
  subjective effects of craving, executive function
- basolateral nucleus of the amygdala
  conditioned cues
- hippocampus (Hippo)
  conditioned contextual cues

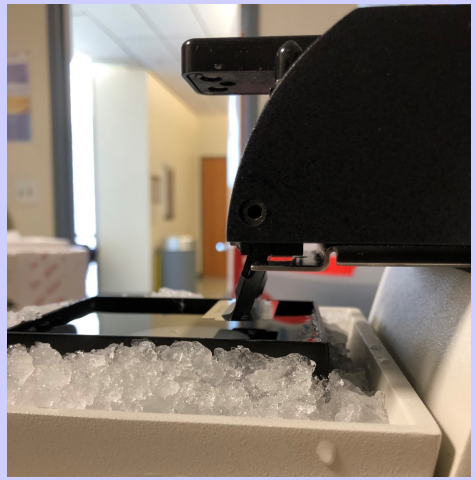
https://pubs.niaaa.nih.gov/publications/arh40/144-151.htm

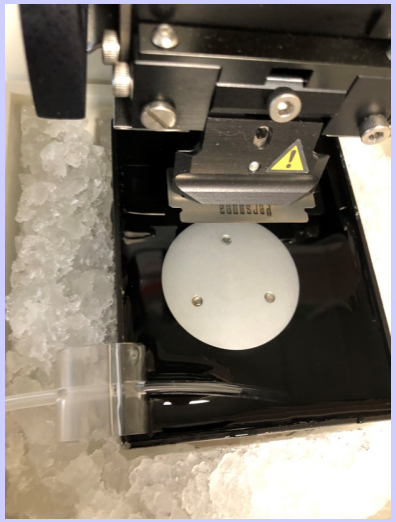
Experimental design

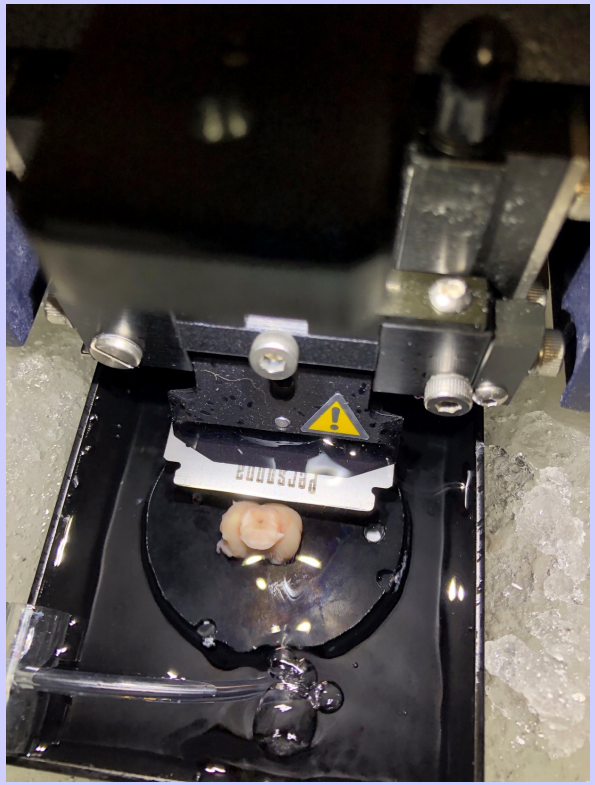
Chronic intermittent ethanol vapor (CIE) – groups are dependent and non-dependent rats

Electrophysiology recordings of firing rate and $GABA_A$ ion channel currents to test differences in neuron signaling between control and ethanol dependent rat brain central amygdala cells

Vibrating microtome set up and brain slicing

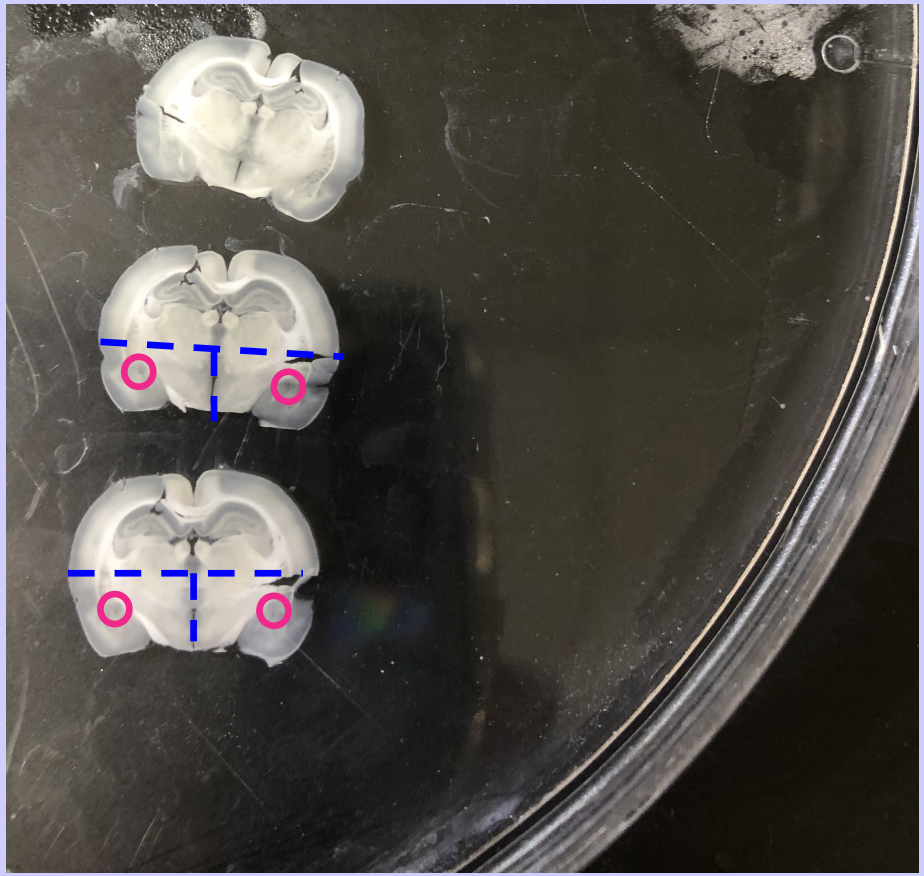


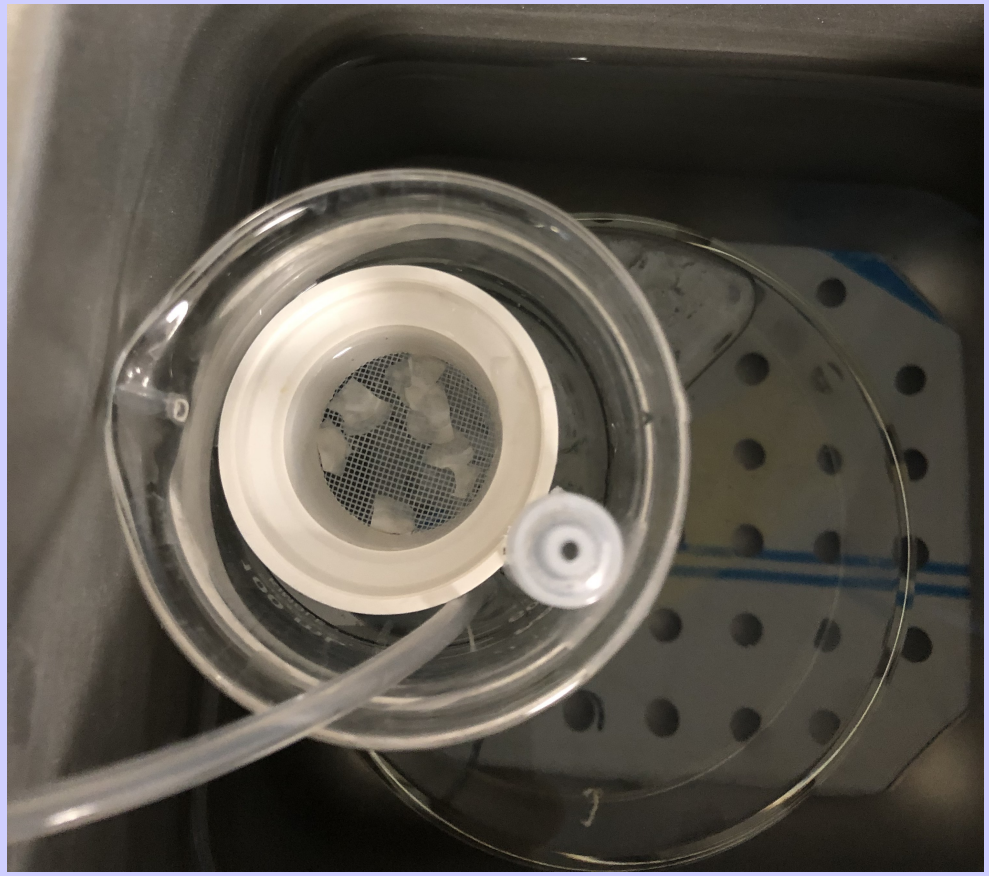




Picture credit: Alicia Avelar, PhD

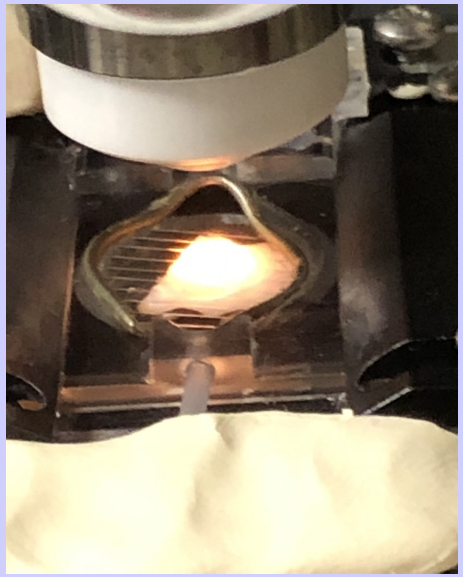
Brain slice trimming and incubation

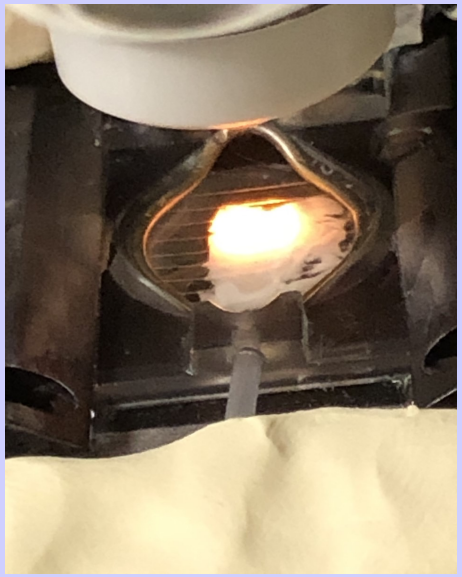


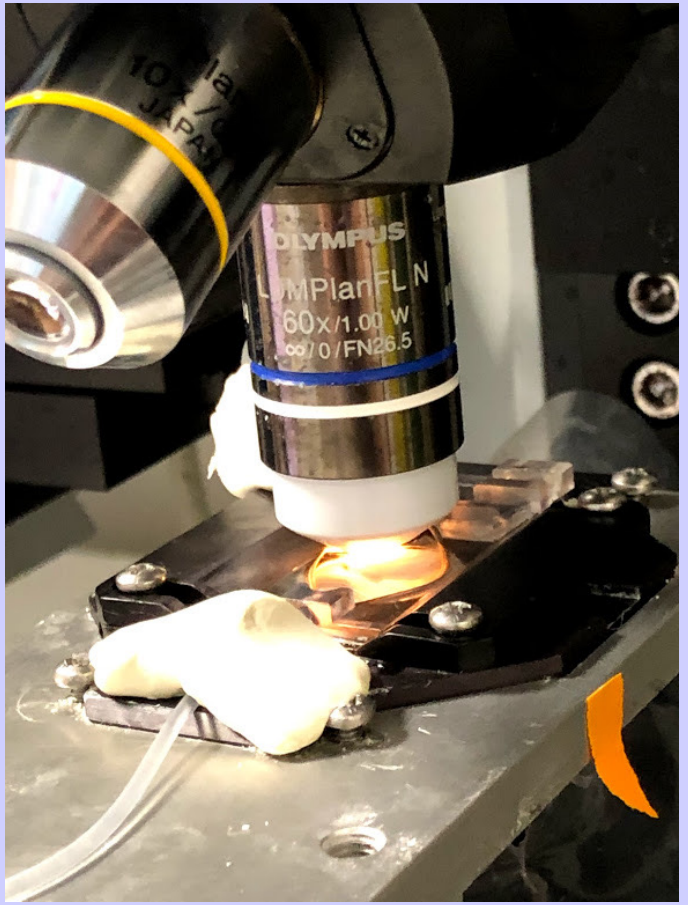


Picture credit: Alicia Avelar, PhD

Rat brain slice placement in the bath

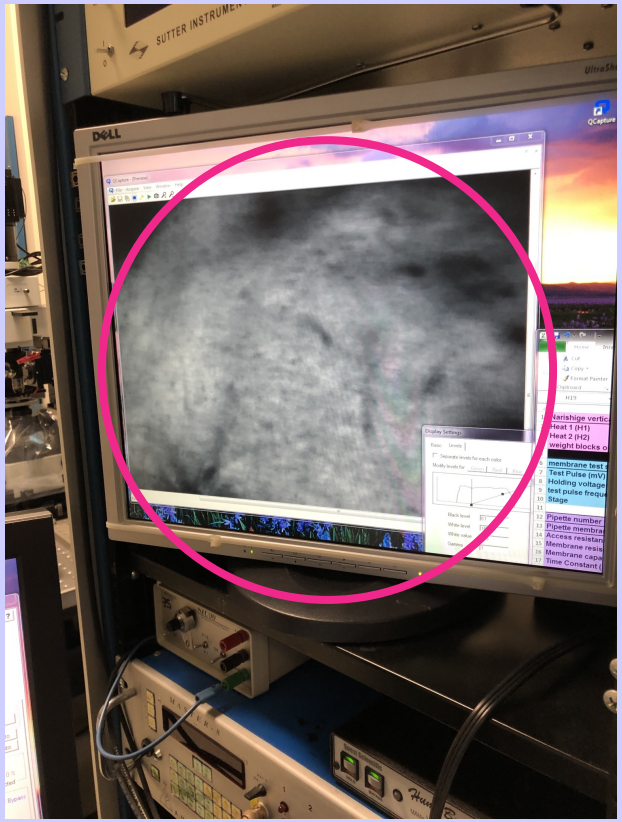


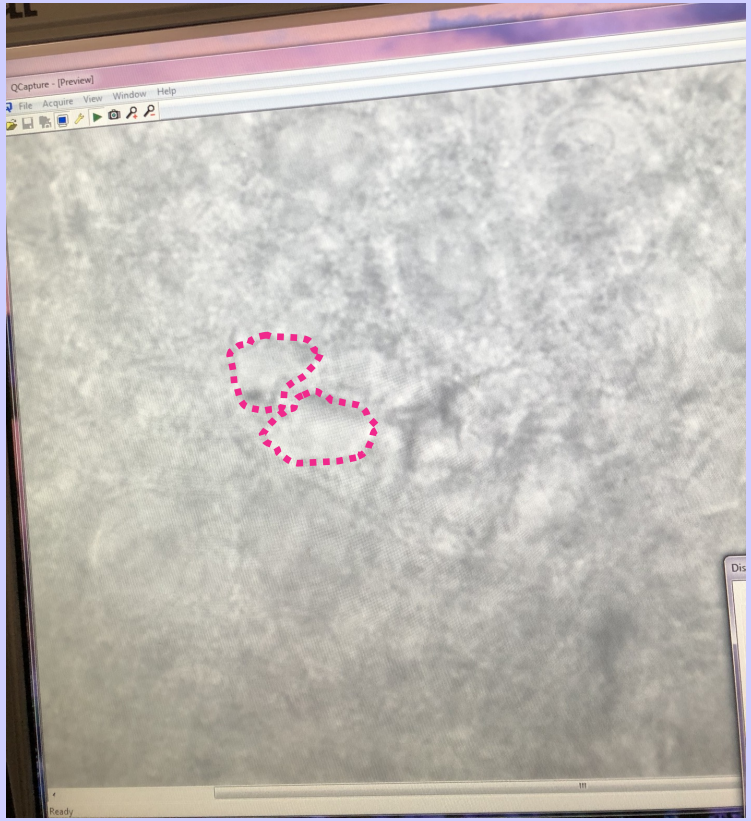


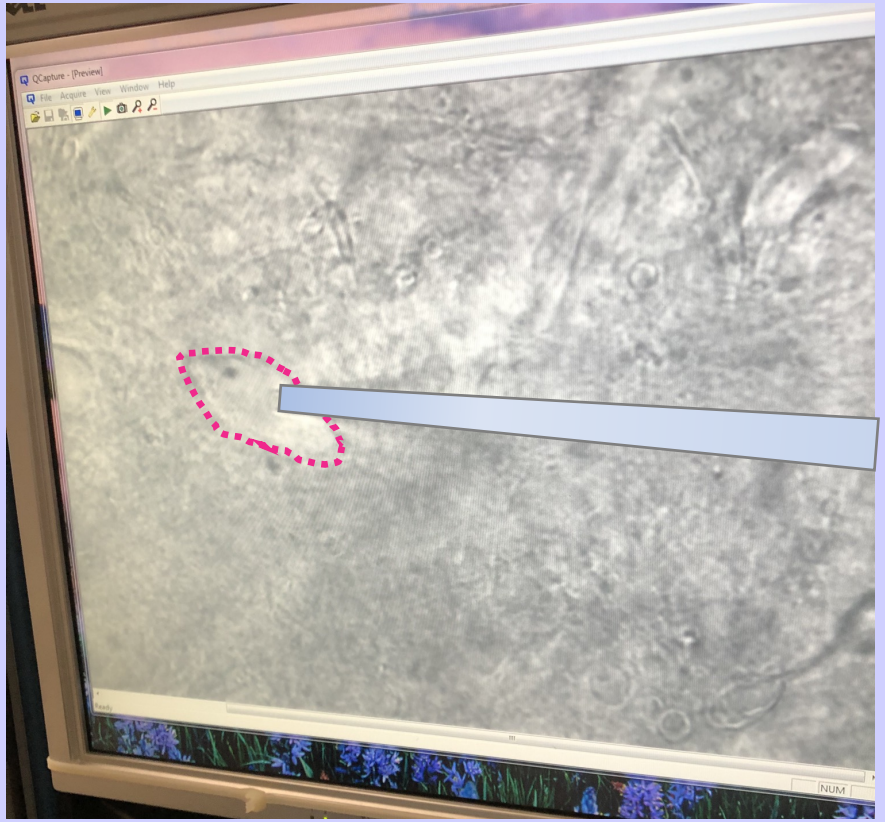


Picture credit: Alicia Avelar, PhD

Central Amygdala (CeA) and GABA cells

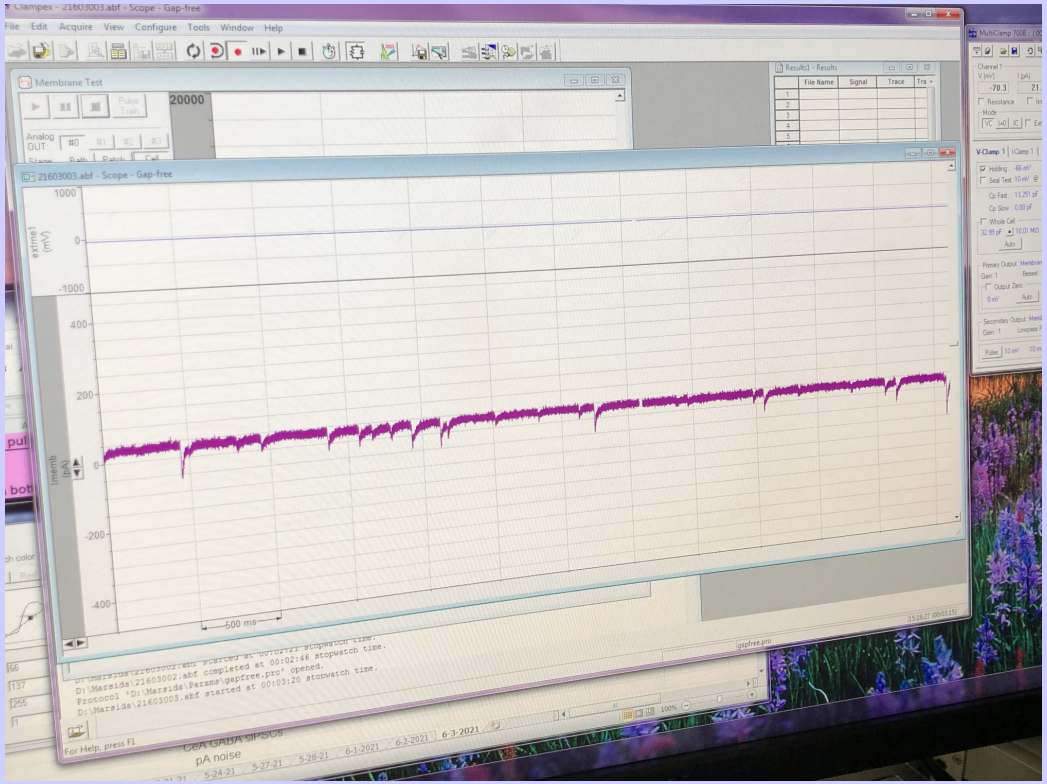


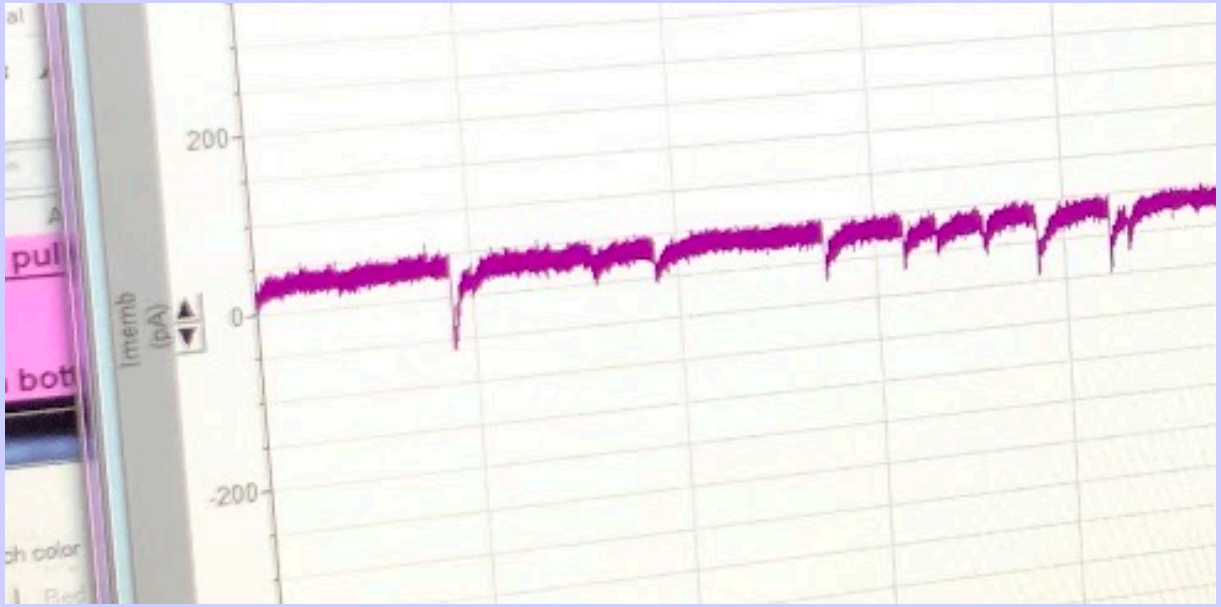




Picture credit: Alicia Avelar, PhD

Closeups of CeA $GABA_A$ spontaneous currents





Picture credit: Alicia Avelar, PhD

Patch-Clamp Electrophysiology video on YouTube, made by scientists

https://www.youtube.com/watch?v=6KoJVkUrv7Q&list=PLn7wDILp3qOUHB1LxYOVMHZWXcdcHDx_S&index=1

Raul Ramos & Emmanuel Rivera-Rodriguez, NBOSS 2021

Jove electrophysiology video

Patch Clamp Electrophysiology | Protocol (jove.com)

References

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- https://www.jove.com/v/5202/patch-clamp-electrophysiology
- https://www.youtube.com/watch?v=Dsq272MUe8M
- https://www.youtube.com/watch?v=HYLyhXRp298
- https://youtu.be/mVbkSD5FHOw
- https://flipper.diff.org/app/pathways/info/3009
- Liss & Roeper, 2008 Brain Research Reviews

Brain slicing and imaging resources

- Precisionary Instruments- https://www.precisionary.com/by-experiment/
- Allen brain atlas- https://mouse.brain-map.org/experiment/thumbnails/100142143?image_type=atlas
- Rat brain atlas - http://labs.gaidi.ca/rat-brain-atlas/
- https://www.olympus-lifescience.com/en/microscopes/upright/bx61wi/#!cms[focus]=cmsContent708
- https://app.biorender.com/user/signin

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