

INTRODUCTION

Alcohol Use Disorder (AUD)

- Chronic relapsing disorder
- Over 80,000 deaths each year
- Effects 17 million people
- The 3rd leading cause of preventable death in the United States
- Costs the US economy \$250 billion/year in direct costs

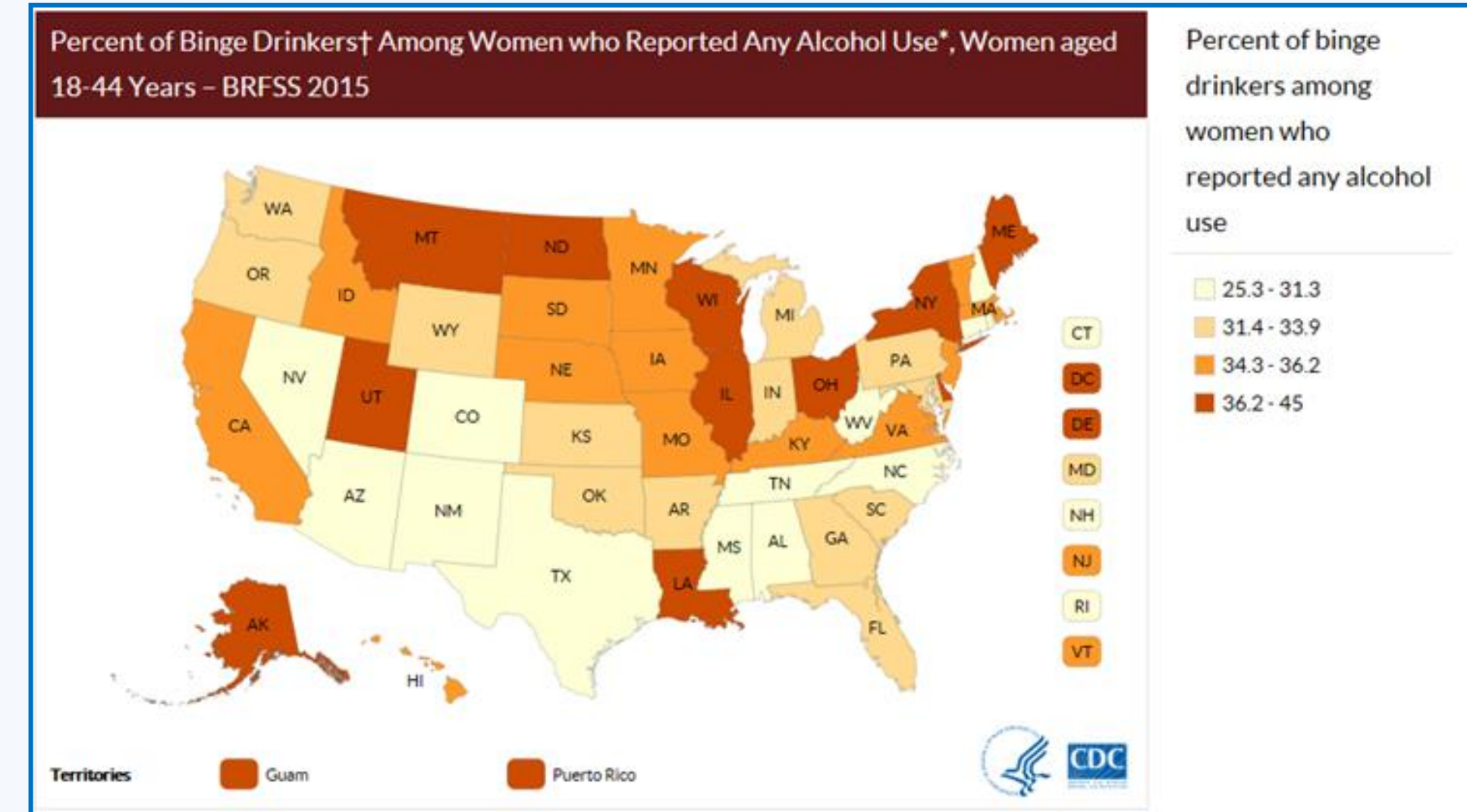


Figure 1. Alcoholism affects women as well as men. Although alcoholism rates are higher in men, women also struggle with AUD (CDC, 2015).

Neurobiology of Addiction

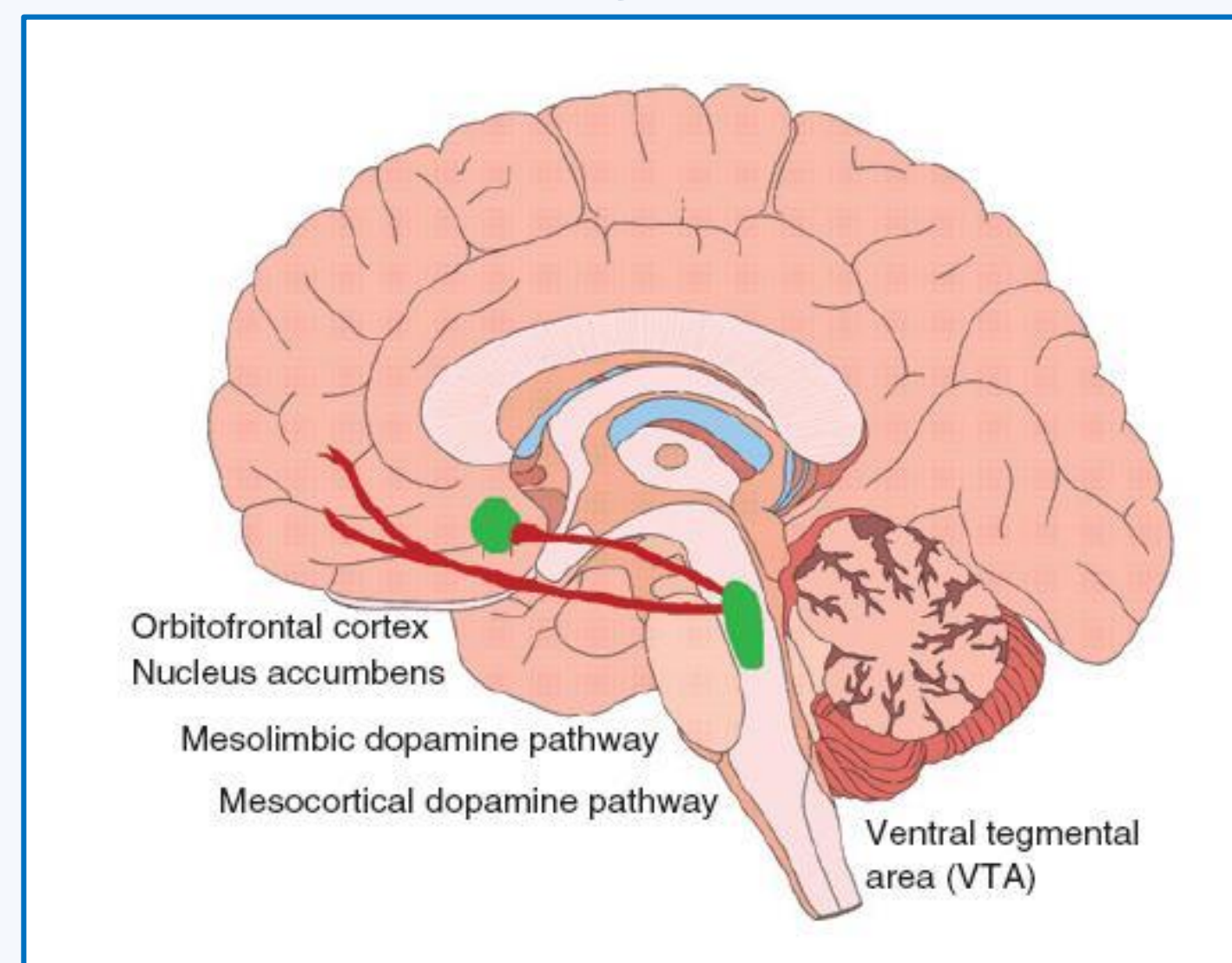


Figure 2. The Mesolimbic reward pathway. Enhanced dopamine (DA) release in the nucleus accumbens (NAc) and enhanced DA neuron activity in the ventral tegmental area (VTA) have been linked to reward, aversion, and associative learning. Ethanol and other drugs are known to enhance DA release in the pathway (Di Chiara, 1988).

HYPOTHESIS

Research goals

- Create an ethanol dosage response curve in male rats measuring basal extracellular DA in the NAc
- Conceptually replicate previous studies showing sex differences in drug-induced DA release in the NAc with ethanol, measuring both evoked and basal response in clinically relevant ethanol dosages
- Measure both evoked and basal release of DA as influenced by ethanol injection in the NAc across the estrus cycle to determine deferential effects

Hypothesis

- There are differential ethanol dosage effects in DA release within the reward circuit
- There are distinct sex differences in DA release following ethanol injection
- Ethanol enhancement of DA release is affected by estrus cycle phase

METHODS

Microdialysis and HPLC with electrochemical detection for dopamine

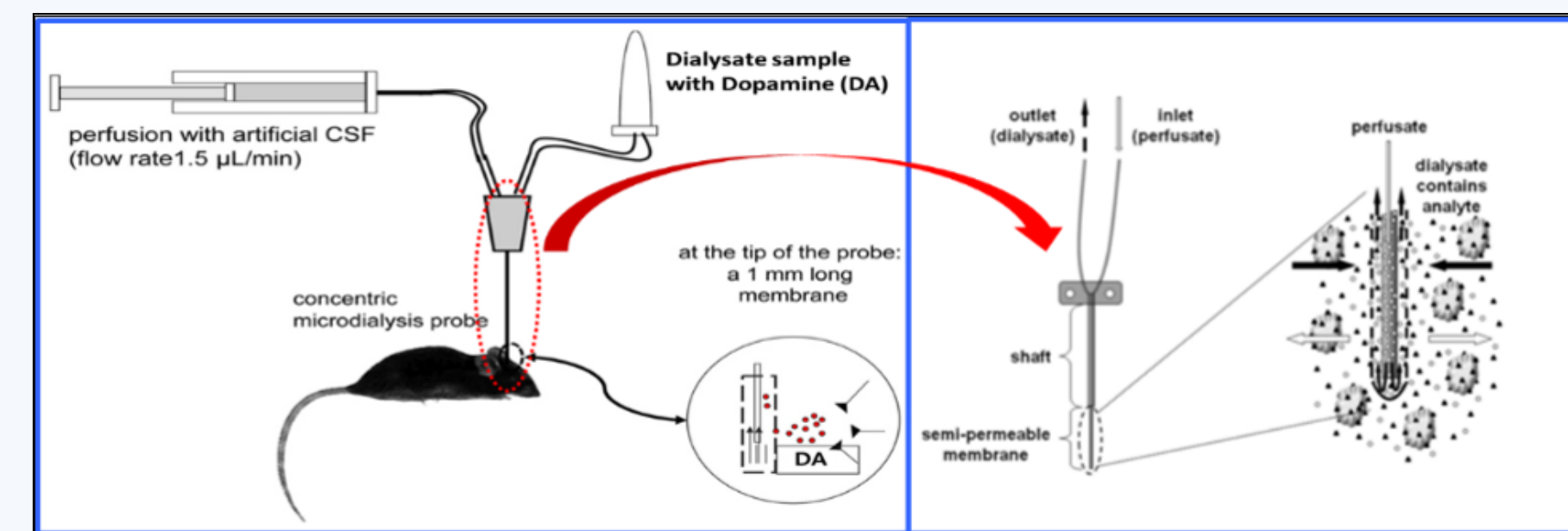


Figure 3. Dopamine microdialysis in the NAc. The *in vivo* microdialysis technique allows measurement of neurotransmitters such as biogenic amines including dopamine (DA). A siliconized guide cannula was stereotactically implanted dorsally to the NAc and then the microdialysis probe was positioned within the guide cannulae into the NAc. The aCSF solution was perfused through the probe by using a microinjection pump at a flow rate of 2.0 µL/min and samples were collected every 20 min. A minimum of six samples were collected to establish baseline neurotransmitter activity before drug administration. After stable controls, rats received ethanol (0.5 – 4.0 g/kg, IP) injections. Nine samples were collect post-injection.

Fast scan cyclic voltammetry for evoked phasic release of dopamine

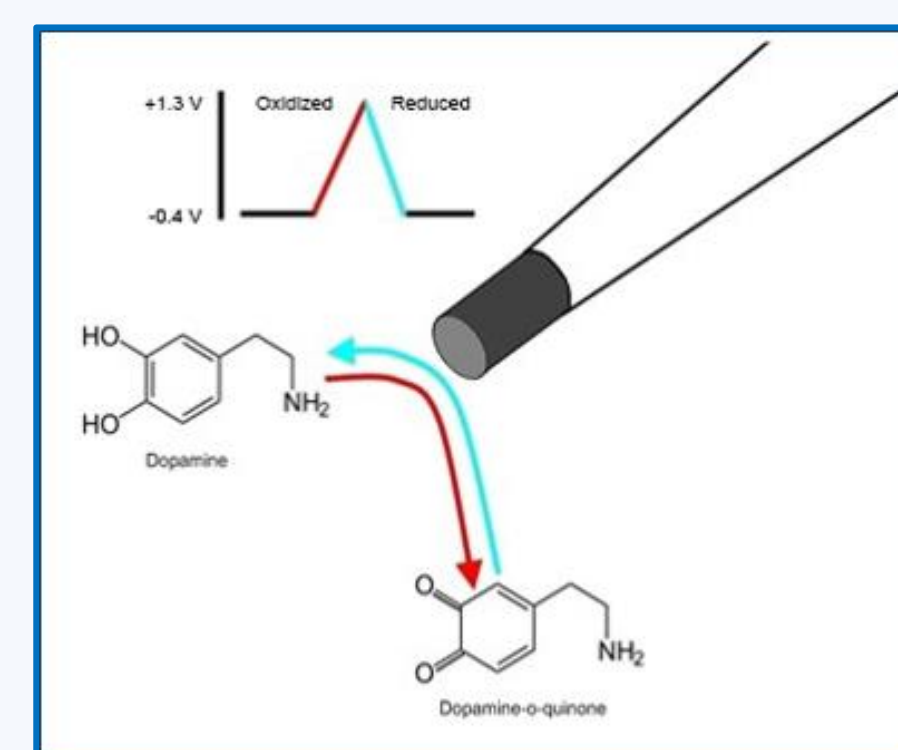


Figure 4. FSCV for evoked phasic release of dopamine. A carbon fiber electrode was stereotactically inserted into the NAc. A second stimulating electrode was inserted into the medial forebrain bundle. A 60 pulse stimulation was delivered to evoke phasic DA release in the NAc. A voltage ramp from -0.4 V to +1.3 V and back was applied to the carbon fiber electrode to facilitate detection of DA.

Vaginal lavages determine estrus phase using Crystal Violet staining

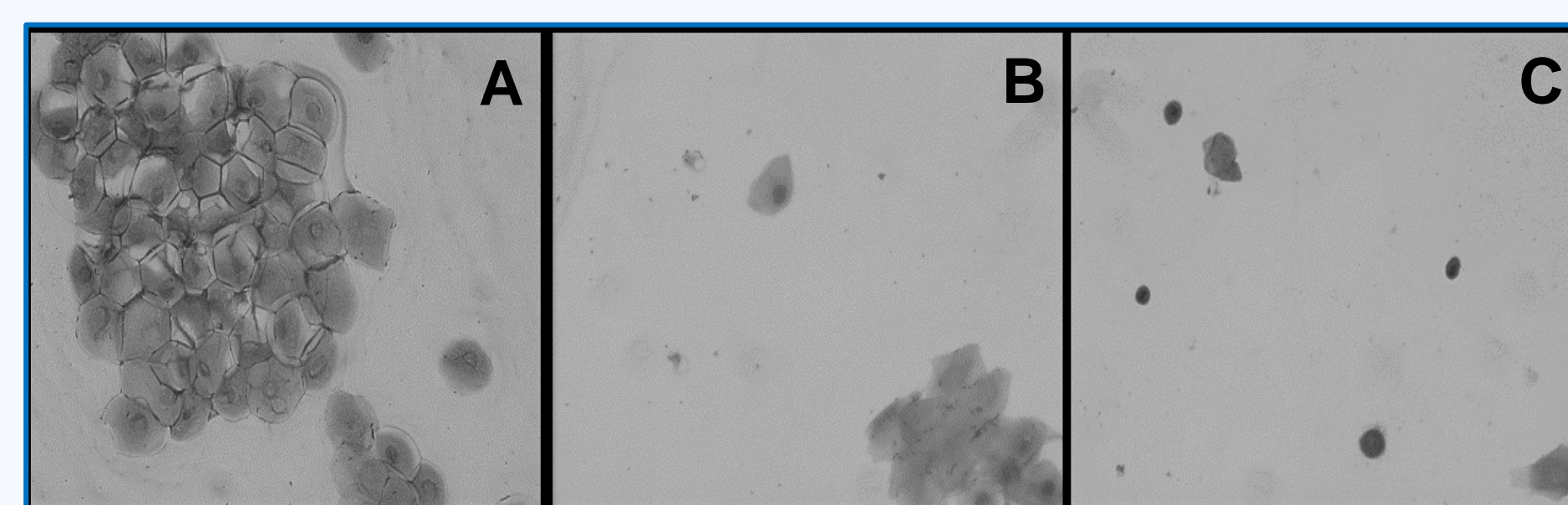


Figure 5. Crystal Violet staining of vaginal lavages. A: Proestrus. Almost exclusively clusters of round, nucleated epithelial cells. B: Estrus. Predominately cornified squamous epithelial cells. C: Metestrus. Mostly small dark leukocytes with some cornified squamous epithelial cells, often fragmented.

RESULTS

Microdialysis: Sex differences in ethanol effects on DA release in the NAc

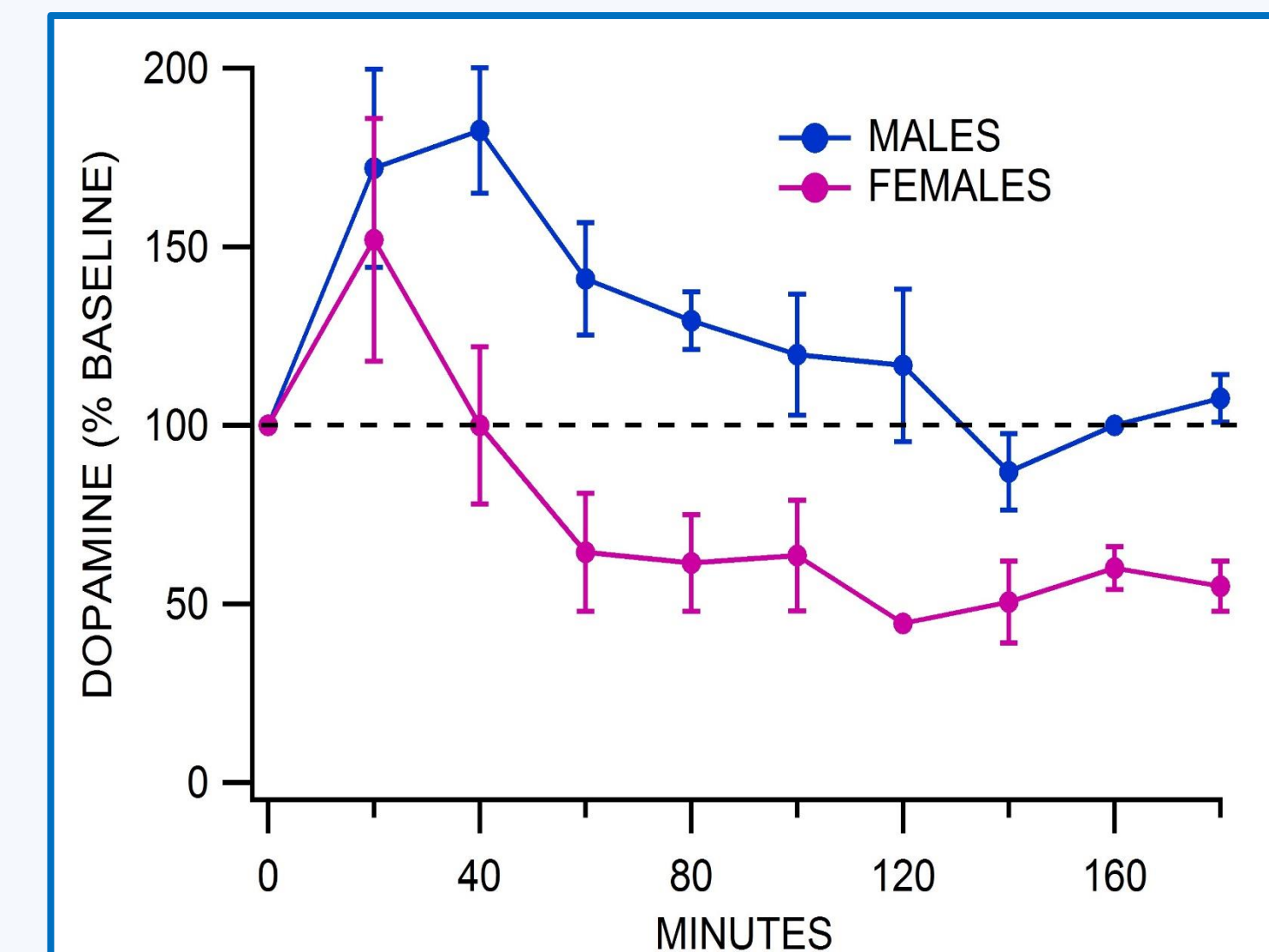


Figure 6. Sex difference in ethanol effects on DA release in the NAc. Preliminary data showing clear kinetic and direction differences between males and females. Although initial basal DA release following injection (2.5 g/kg, IP) is similar, females rapidly declined in DA release and undershot their baseline dopamine following ethanol while males did not (Males: n=8; Females: n=3).

Microdialysis: Dosage Response curve of ethanol on DA release in NAc

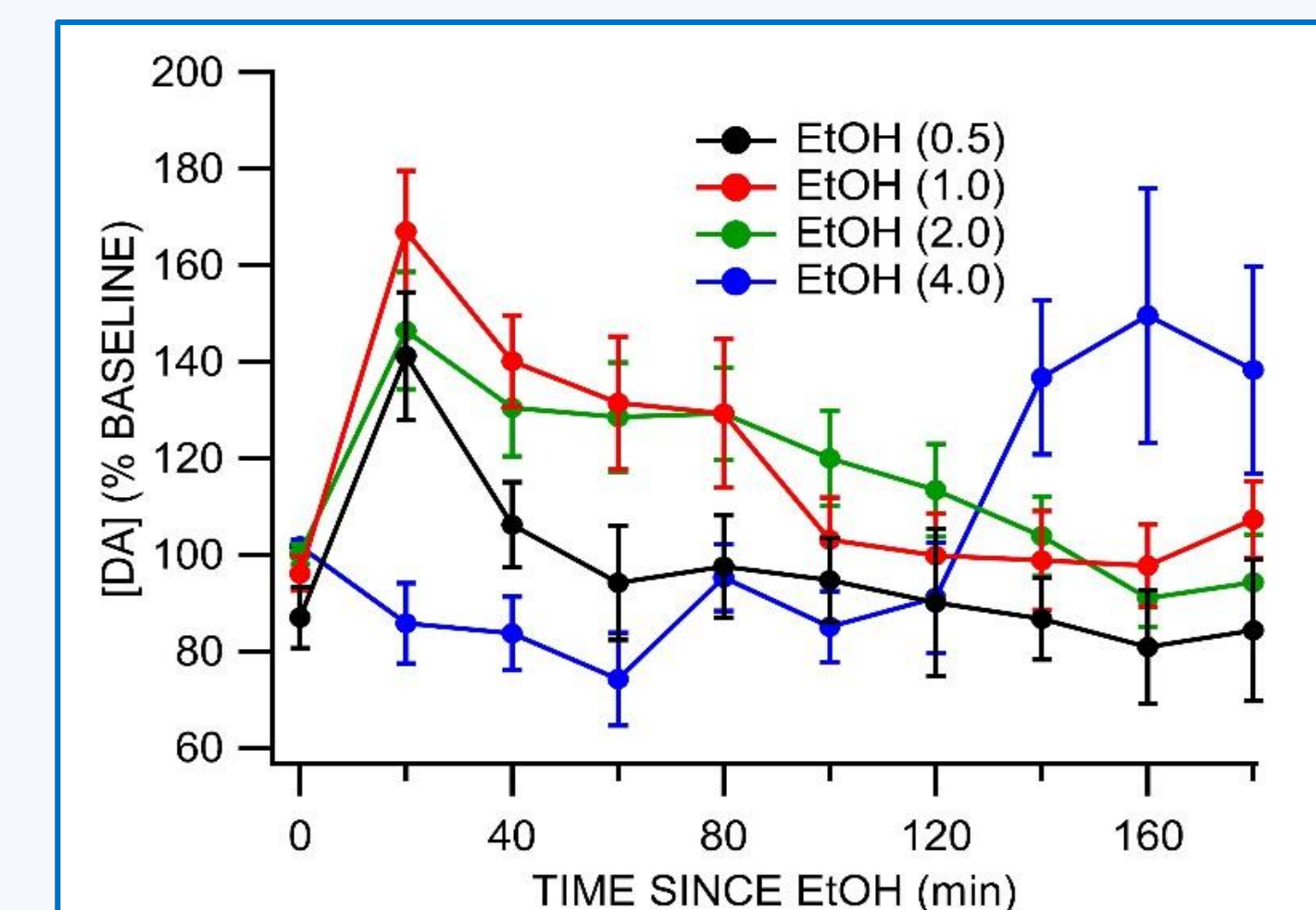


Figure 7. Effect of ethanol on basal extracellular DA release in the NAc of male rats. Ethanol enhances DA release in the NAc in a dose-dependent manner.

FSCV: Sex Differences in ethanol effects on DA release in NAc

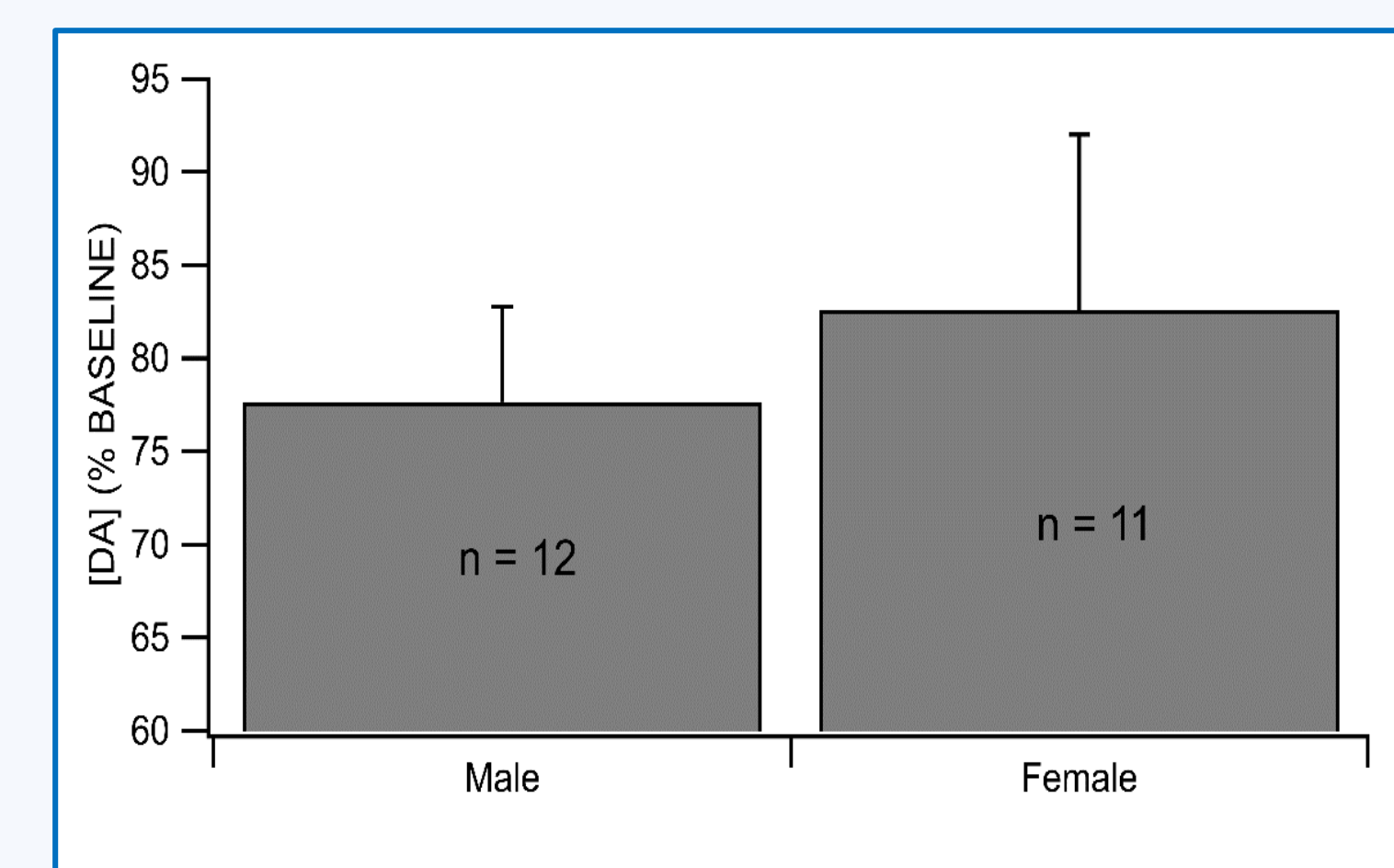


Figure 8. Lack of sex effects for ethanol inhibition of dopamine release in the nucleus accumbens. Acute ethanol (2.0 g/kg, IP) reduces evoked DA release in both males and females 20 min after injection.

RESULTS (cont'd)

FSCV: Effects of ethanol on evoked DA release in the NAc across estrus cycle

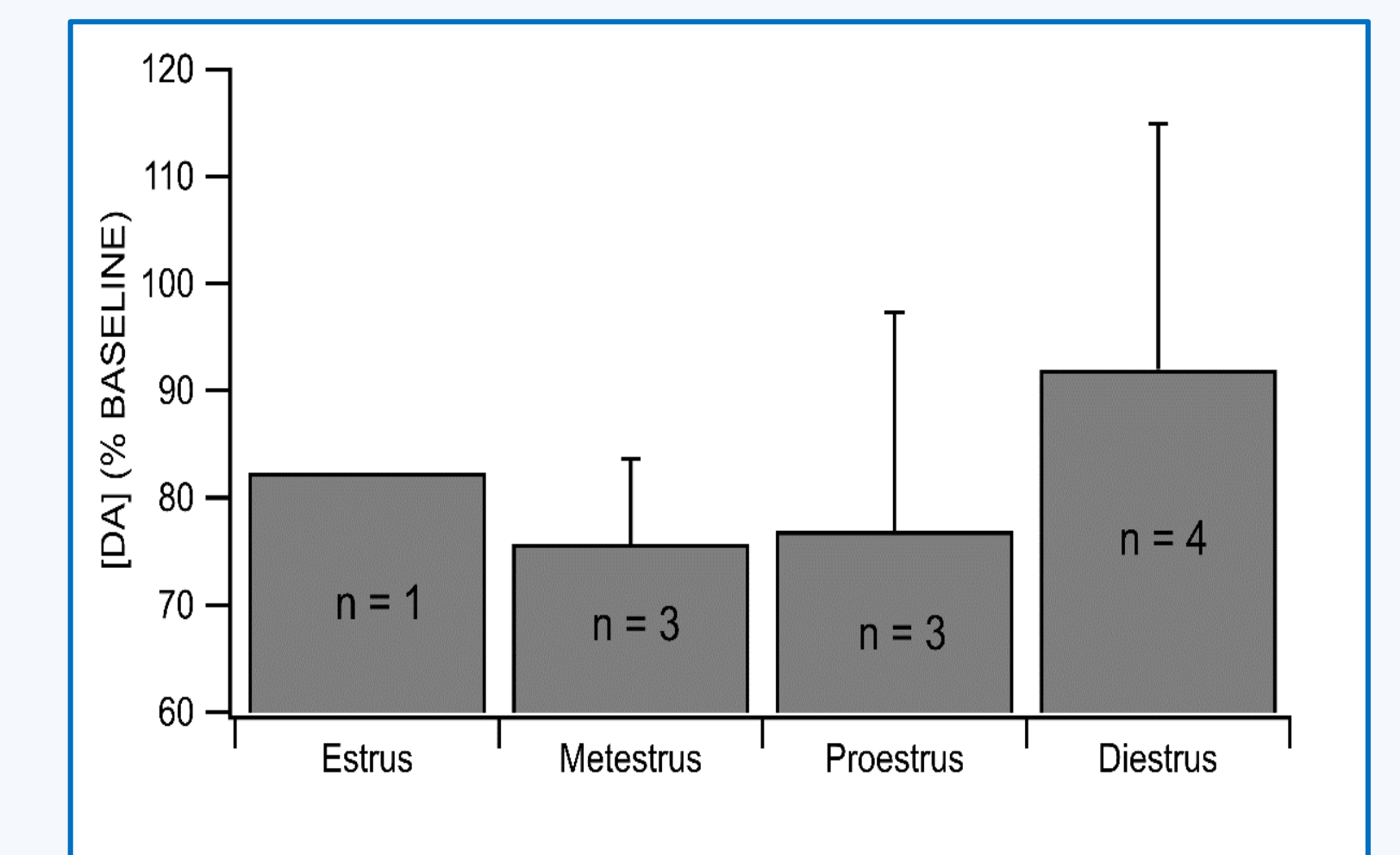


Figure 9. Preliminary data showing effects of ethanol on evoked dopamine release in the nucleus accumbens across phases of the estrus cycle. Acute ethanol (2.0 g/kg, IP) reduces evoked DA release but with no apparent differences across phases of the estrus cycle.

SUMMARY AND CONCLUSIONS

- There are distinct sex differences in basal extracellular DA release due to ethanol including an undershot of baseline after acute ethanol injection in females.
- Ethanol enhances basal extracellular DA release in the NAc in a dose-dependent manner in male rats.
- There are no apparent sex effects for ethanol inhibition of evoked dopamine release in the NAc, but experiments are ongoing.
- There are no apparent differences in ethanol induced evoked DA inhibition across the estrus cycle. Sample sizes are still low and experiments are ongoing.
- Due to recently lengthy renovations of the KMBL vivarium and our HPLC being down, female microdialysis experiments are just now beginning to be run and analyzed. Data for this is ongoing and looks promising.
- Conclusions of no effect between males and females and estrus phase differences will still aid understanding of sex differences and will benefit the field as it complies with recent NIH sex studies requirements.

ACKNOWLEDGEMENTS

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