Prenatal Alcohol and Cannabinoid Exposures Disrupt Acetylcholine Receptor Subunit Expression in Adult Mouse Offspring

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Fetal Alcohol Spectrum Disorders (FASD)

FASDs can present through a variety of behavioral symptoms, including:

- **Difficulty controlling emotions and impulsivity**
- **Depression and anxiety**
- **Attention deficit/hyperactivity disorder (ADHD)**
- **Motor coordination impairments**
- **Alcohol seeking**

However, not every individual with FASD will experience these symptoms. Factors like sex and additional drug exposure may also play a role.

How Does Prenatal Alcohol and Cannabinoid Exposures:

1

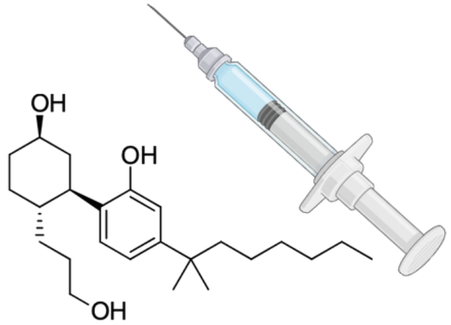
Disrupt Offspring Behavioral Profiles

Prenatal Exposure Paradigm

F0: Adult pregnant female C57B1/6J mice

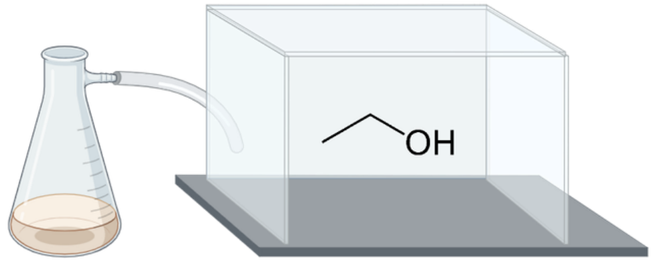
Assign Offspring to Exposure Groups

1. **CB-/ALC-** Controls
2. **CB-/ALC+** Alcohol exposure
3. **CB+/ALC-** Cannabinoid exposure
4. **CB+/ALC+** Dual exposure



Cannabinoid exposure: I.P. injection of cannabinoid agonist **CP-55940** (750 µg/kg, in 10% DMSO/Saline) or **10% DMSO in Saline** (Control)

AND/OR

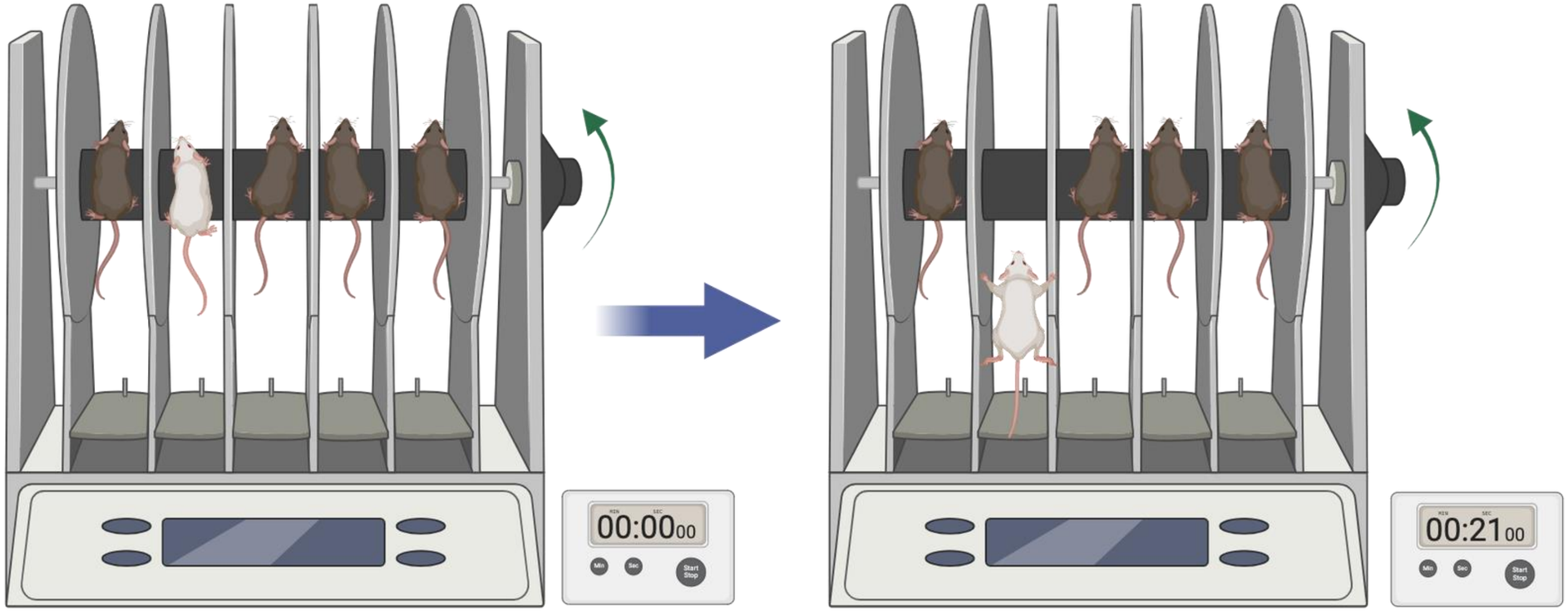


Alcohol exposure: 30-minute inhalation period of **95% Vapor Ethanol** or **Room Air** (control)

Gestational Day (G)12-15: Daily drug exposures at 9AM

On postnatal day (P)100+, offspring underwent: 1) Rotarod Test for motor coordination, 2) Open-Field Test (OFT) for anxiety-like behaviors and hyperactivity, and 3) Operant self-administration of ethanol for various alcohol-seeking behaviors.

The rotarod test assesses motor coordination by placing the mice on an accelerating, rotating rod and measuring the time they can balance before falling off.

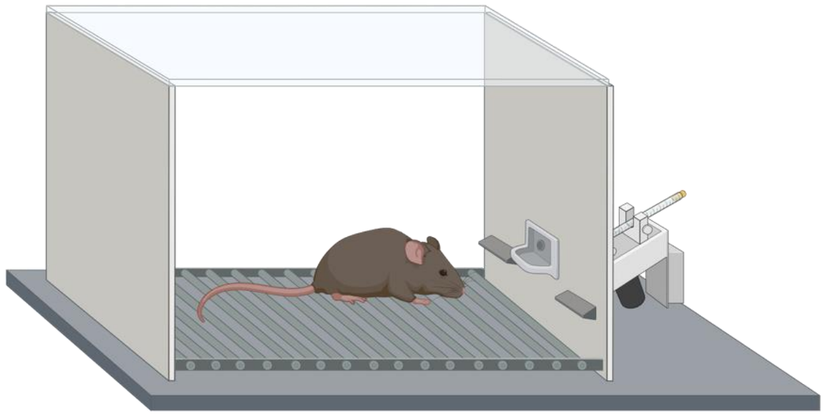


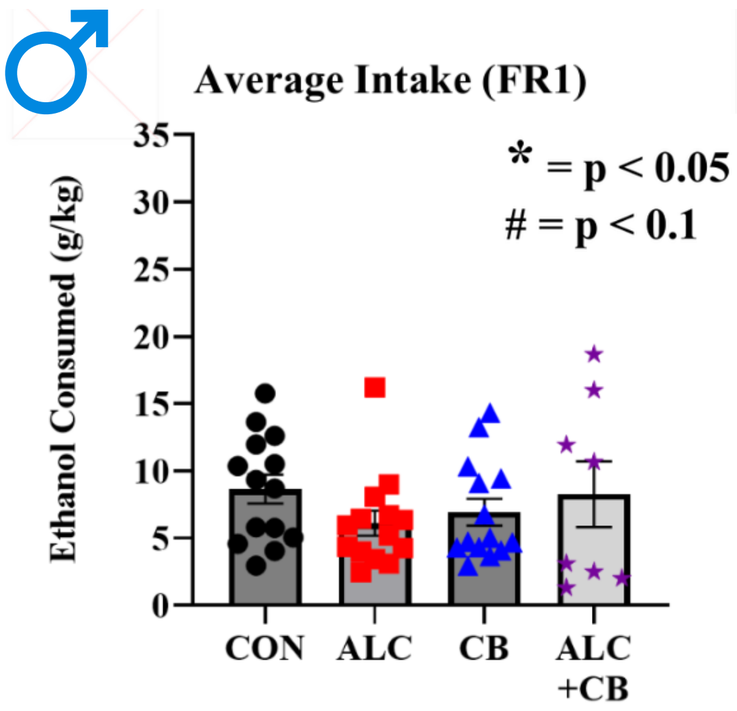
Shorter times spent on the rotarod indicate decreased motor coordination.

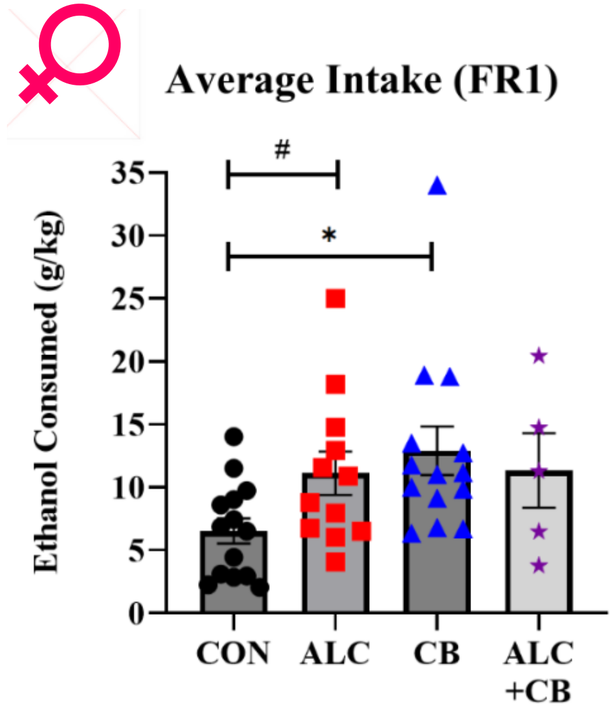
In males, all exposures decreased the time spent balancing on the rod compared to controls.

In females, ONLY single drug exposure decreased the time spent balancing on the rod.

The operant chamber protocols (FR1 and PR) assess how different prenatal exposures to single and dual substances can affect aspects of alcohol-seeking behavior.

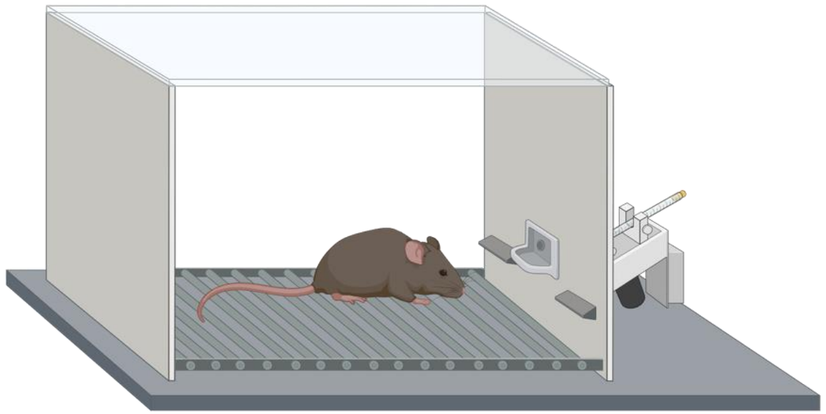


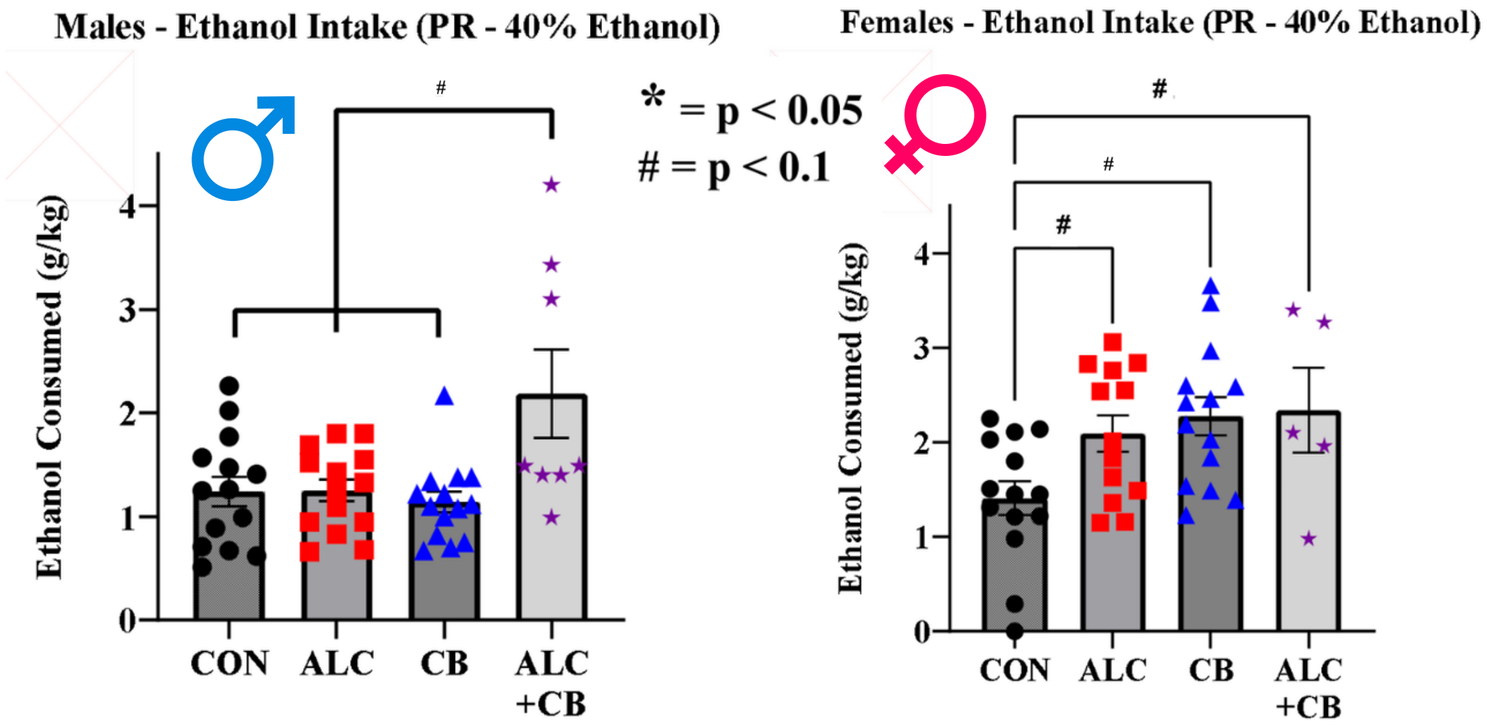




In males, there is no difference in how much subjects drank based on exposure.
In females, when subjects received either alcohol exposure or cannabinoid exposure, they drank more.

The operant chamber protocols (FR1 and PR) assess how different prenatal exposures to single and dual substances can affect aspects of alcohol-seeking behavior.





Dual exposure in both males and females increased ethanol intake compared to controls.

Prenatal alcohol and/or cannabinoid exposures impose sex-specific motor deficits and alcohol-seeking behavior in adult offspring.

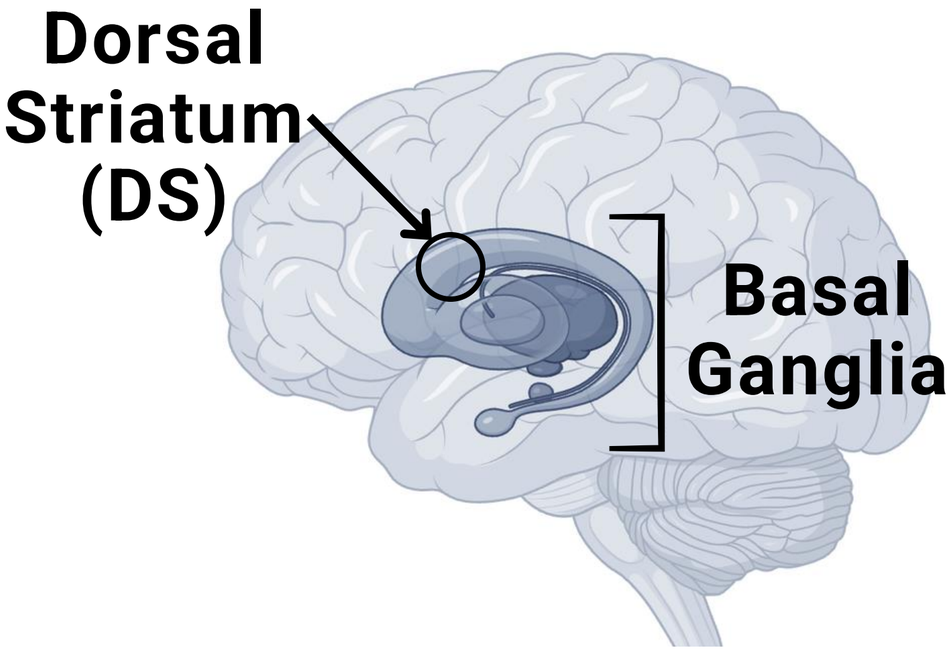
	ROTAROD TEST		OPERANT CHAMBERS (ALC SEEEKING)			
	MOTOR COORDINATION		FR1		PR	
	M	F	M	F	M	F
CONTROL						
ALC	↓	↓		↑		↑
CB	↓	↓		↑		↑
ALC + CB	↓				↑↑↑	↑

↑↓: Significant difference from controls ↑↓: Significant difference from PAE ↑↓: Significant difference from PCE

Acetylcholine Nicotinic Receptors

What is Acetylcholine (ACh)?

- **ACh is a key neurotransmitter that acts as a messenger. ACh transmits critical information about FASD-like behaviors, such as learning, motor coordination, and memory, throughout the brain - notably in the Dorsal Striatum.**

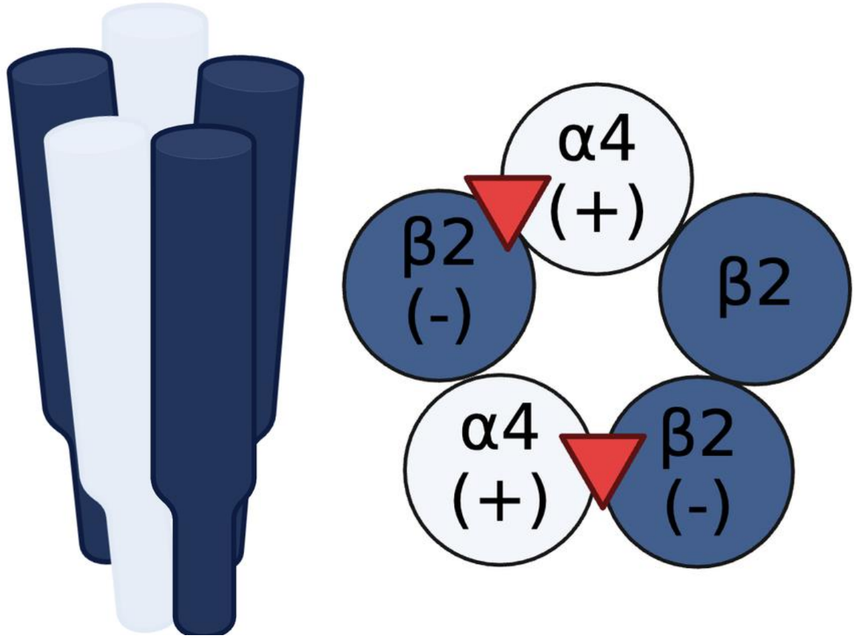


What are Nicotinic Receptors (nAChRs)?

- **There are two families of ACh receptors: Nicotinic and Muscarinic. Nicotinic Receptors are composed of 17 sub units that rearrange to create different combinations.**

Why do nAChRs matter?

- **nAChR-α4β2 is a notable nAChR, and it has a heteromeric structure. CHRNα4 and CHRNβ2 are genes that encode the nAChR-α4 and nAChR-β2 subunits. nAChR-α4β2 plays a key role in motor control and reinforcement-based learning.**



How Does Prenatal Alcohol and Cannabinoid Exposures:

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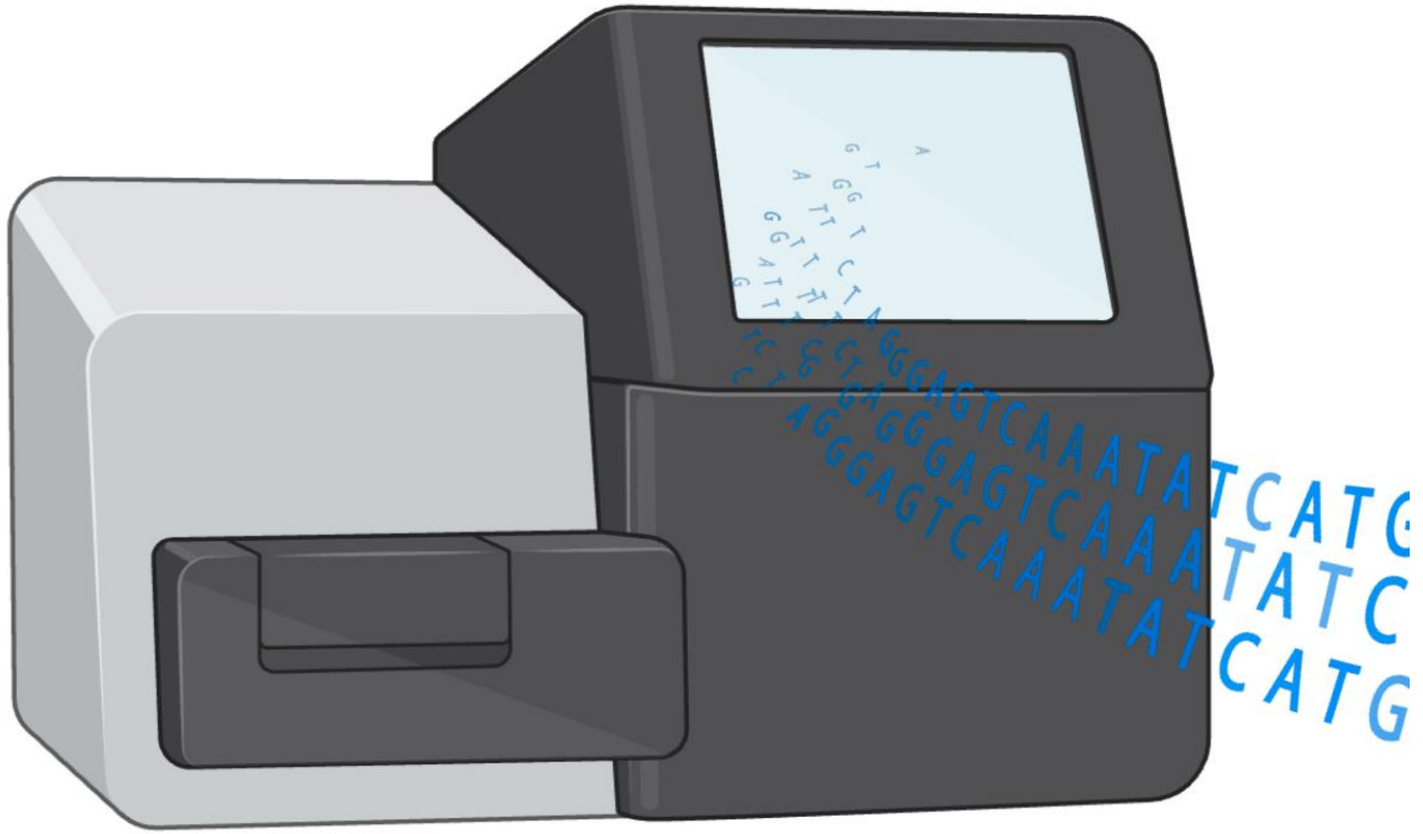
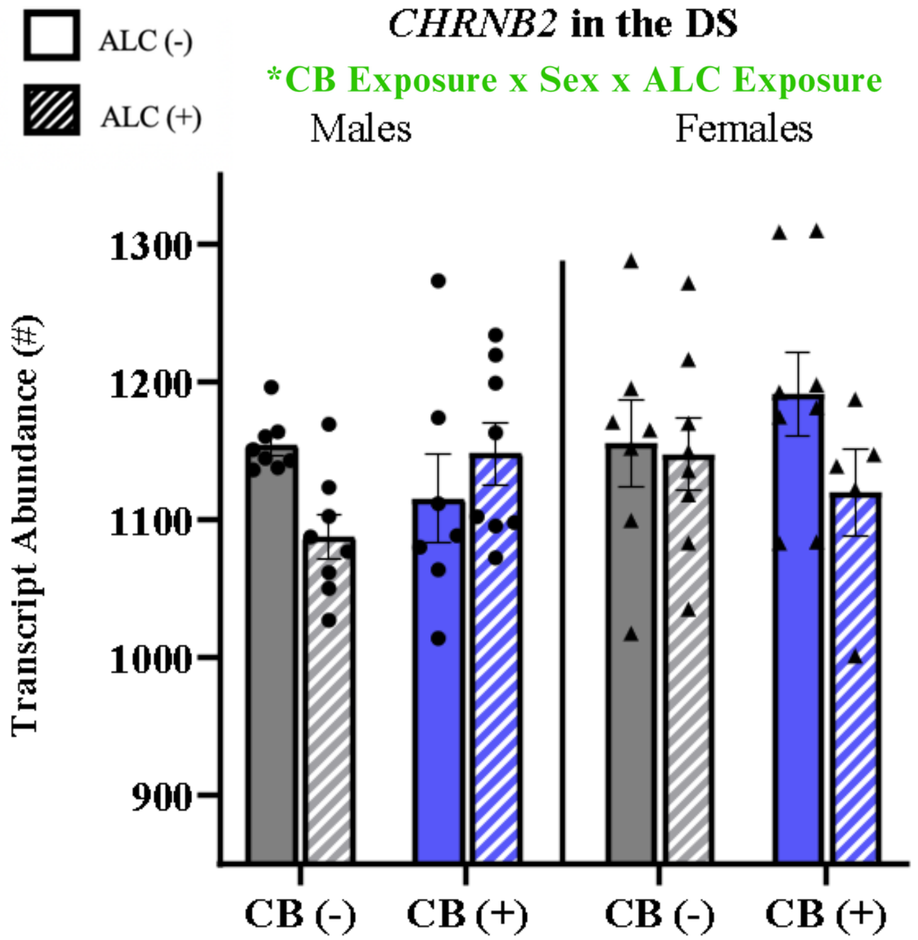
Impact Gene Expression

After behavioral testing, at ~1 year of age, offspring were euthanized for tissue collection of the Dorsal Striatum and RNA-sequencing was performed to determine the level all gene expressions.

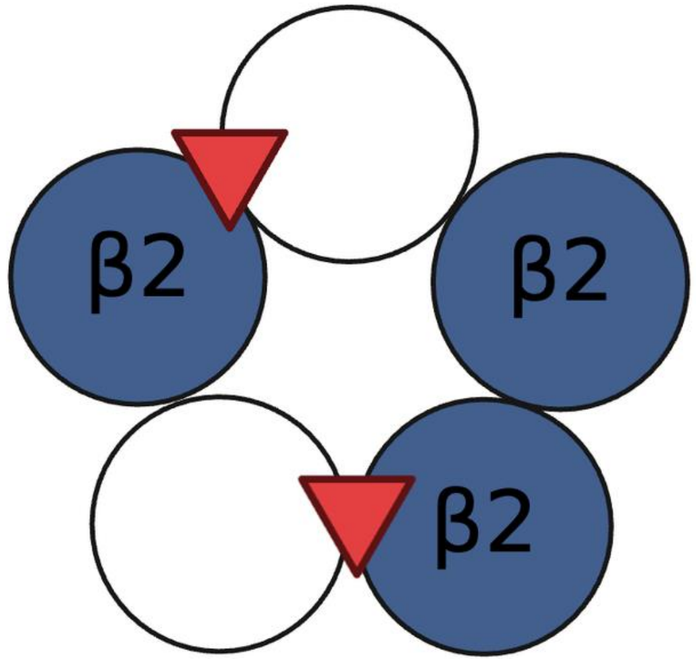
- Total RNA Isolation: Trizol
- Gathering Raw FASTQ Files:Illumina Stranded Total RNA Library Preparation and Sequencing
- Quality control: FASTQ
- Pre-processing: Trimmomatic
- Read alignment: HISAT2
- Transcript Quantification: HTSeq
- Expression analysis



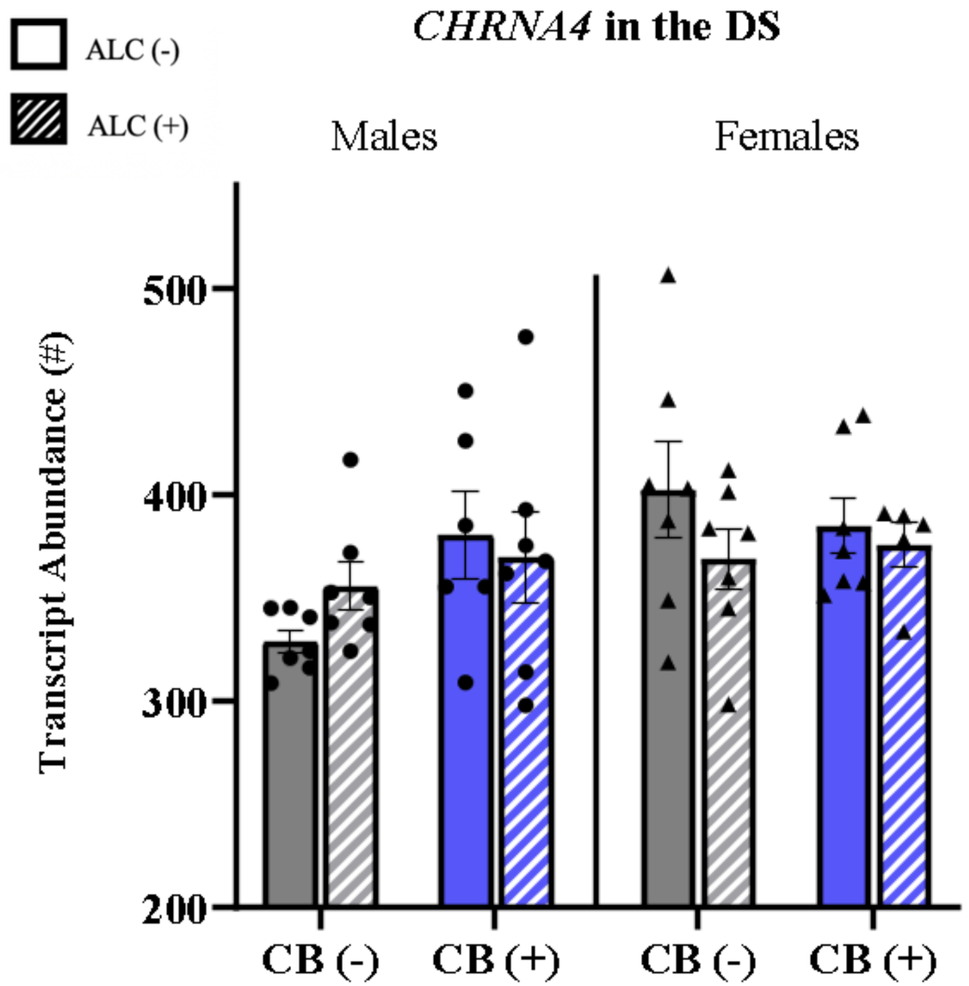
3 way interaction of CB exposure, ALC exposure, and SEX was observed in CHRNB2 in the DS.



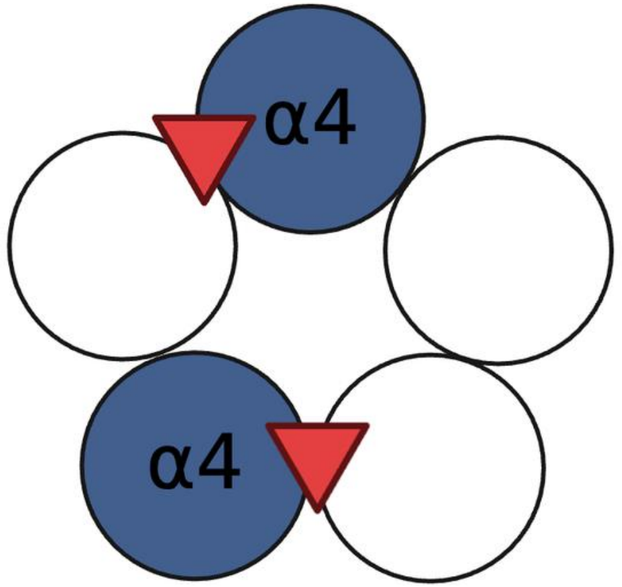
CHRNB2 top view



Sex Differences: Females, regardless of exposure, had more α4 expression in the DS.



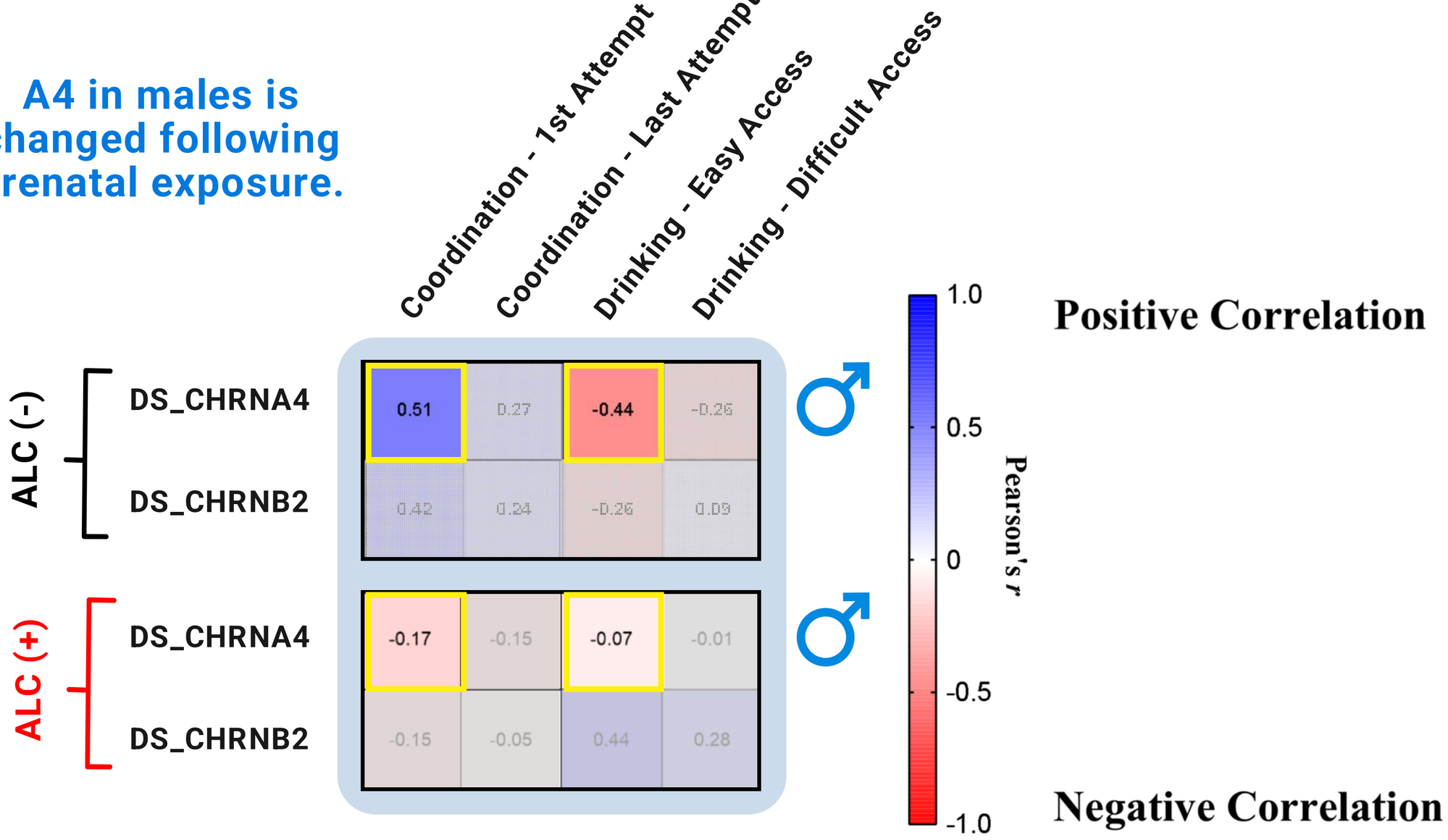
CHRNA4 top view



CAN THE EXPRESSION OF GENES SERVE AS A BIOMARKER OF BEHAVIORAL PHENOTYPES?

3

A4 in males is changed following prenatal exposure.



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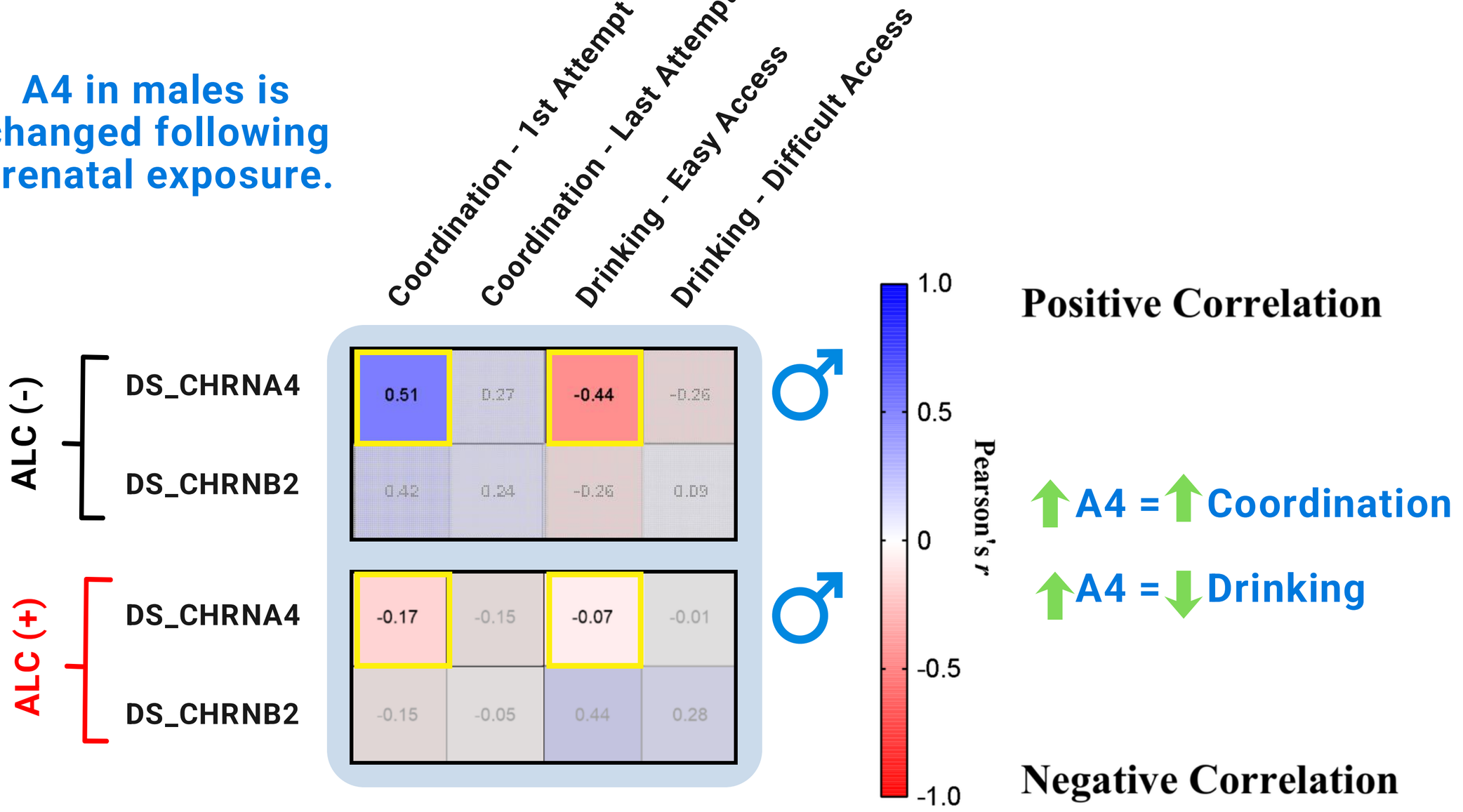

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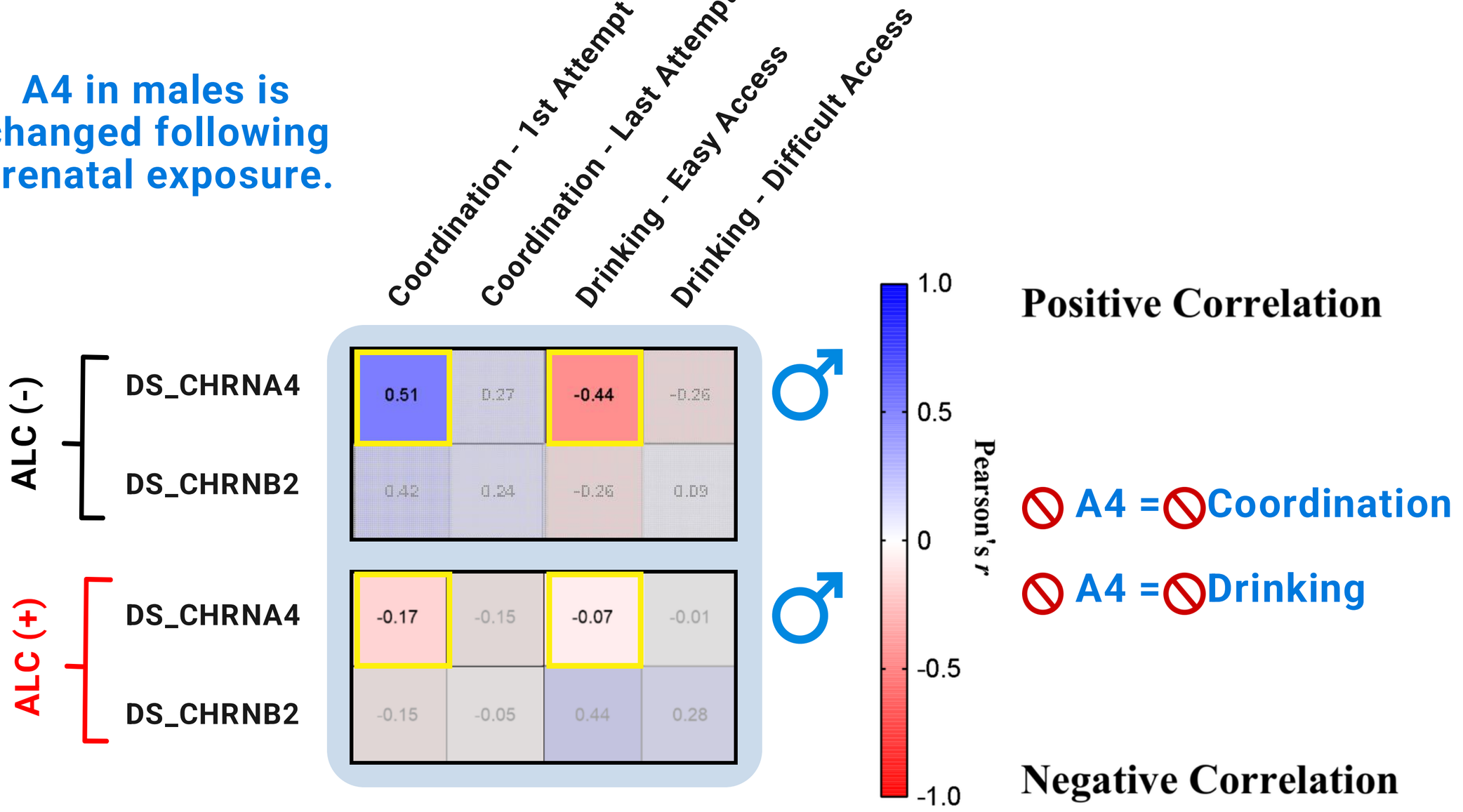
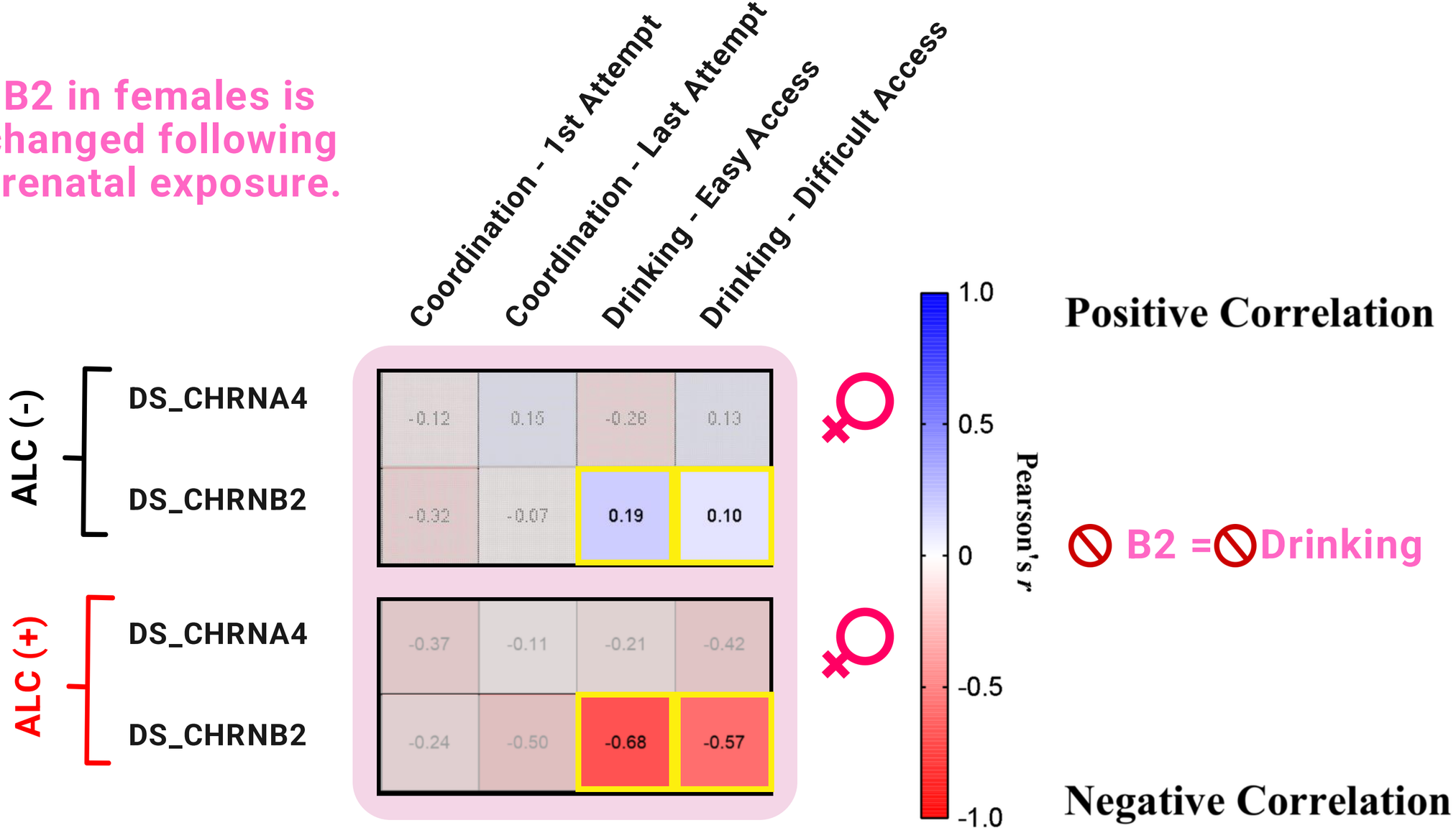


A4 = Coordination

A4 = Drinking

B2 in females is changed following prenatal exposure.



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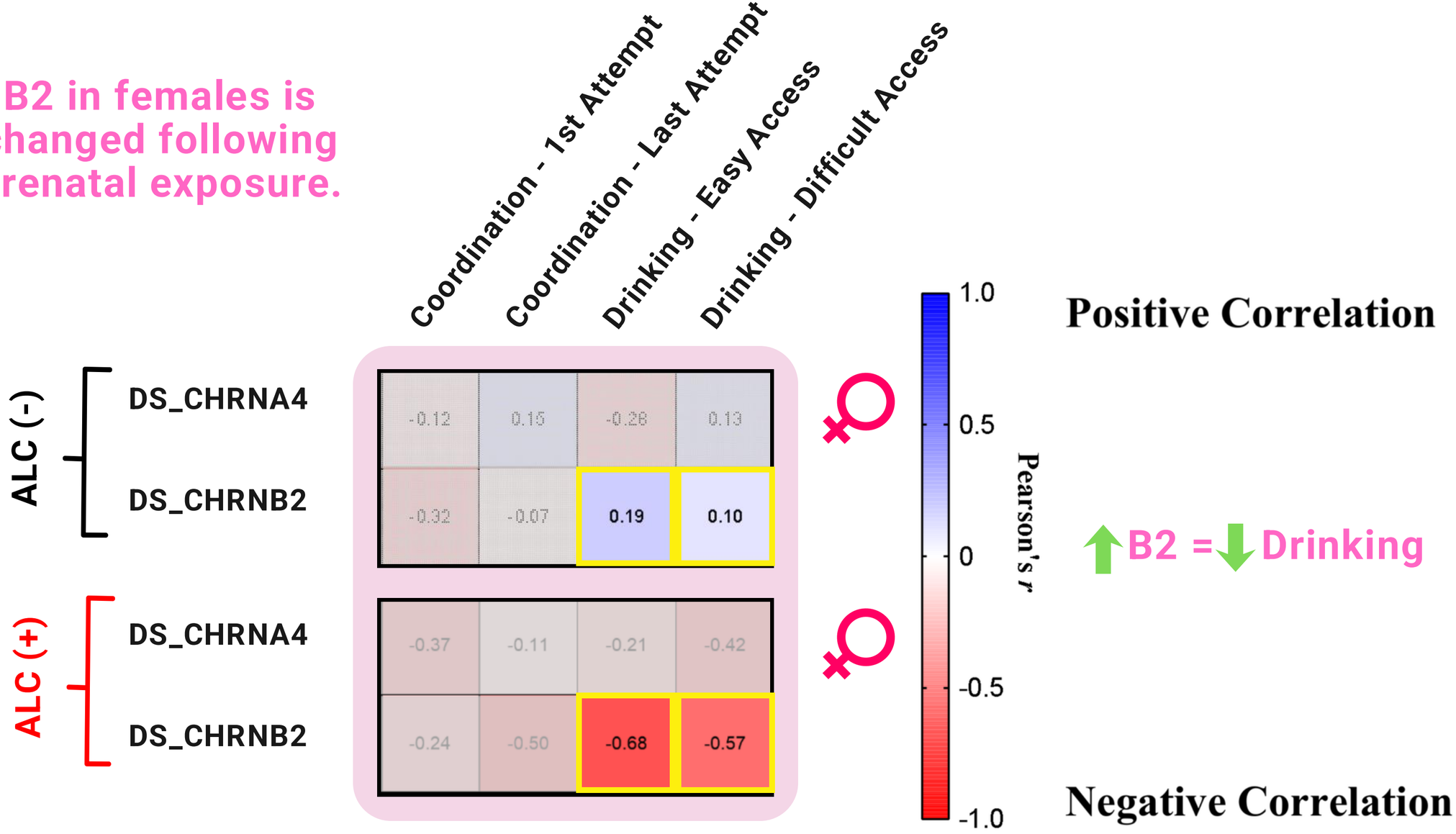


CONCLUSION

1

Replicated behaviors associated with prenatal exposure in humans in mouse offspring

2

Observed sex and exposure specific changes in the expression of subunits a4b2

3

Noted subunit expression corresponds with offspring behavior in a sex and exposure

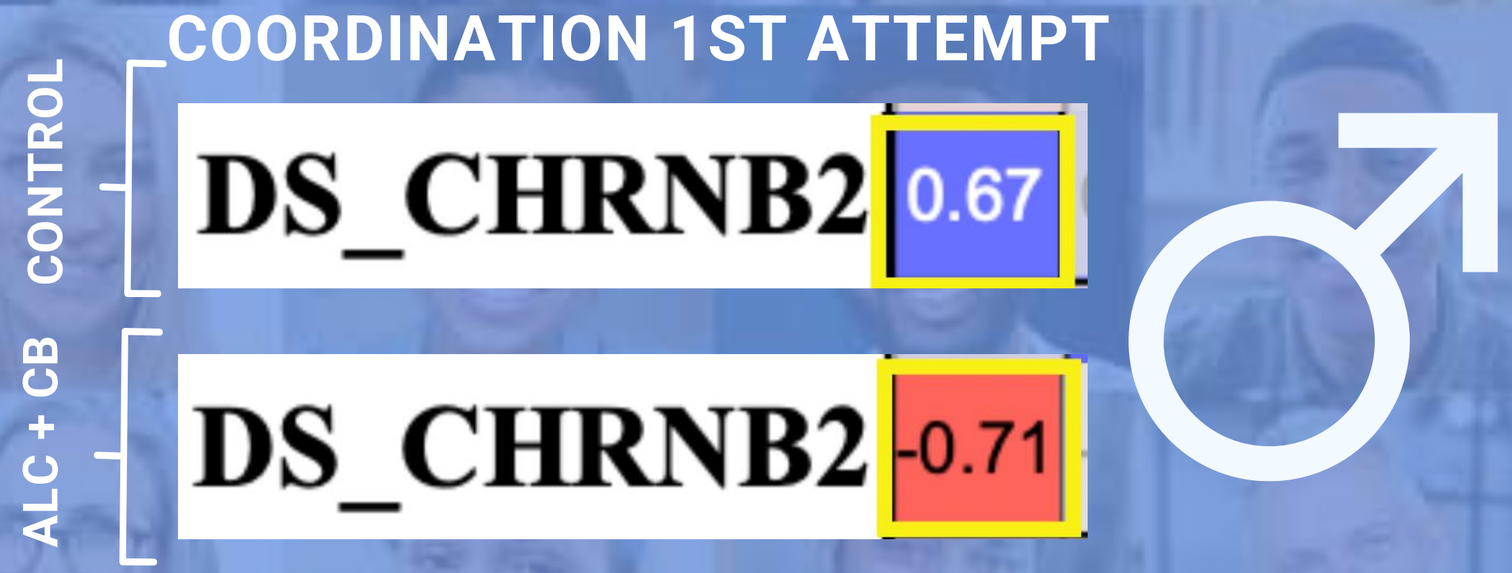
Its important that we consider polysubstance exposure as well as sex while assessing FASD symptoms.

Dual exposure produces distinct outcomes compared to single exposure

FUTURE DIRECTION

In this study, we verified that **prenatal exposure to alcohol and/or cannabinoids produces FASD-like behaviors**. Moving forward, our next questions are:

How can we target these system to correct the observed outcomes AND take into account the conflicting effects of different prenatal substance exposures and sex?



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All graphics were made using BioRender.com.

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