Early life maternal separation enhances stress-induced alcohol abuse vulnerability in male and female msP rats

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Alcohol use is a large public health problem

- Alcohol use account for ~5% of the global disease burden, and ~5% of all deaths (3M/yr)
- A large part of the disease burden from alcohol is associated with alcohol use disorder (AUD).

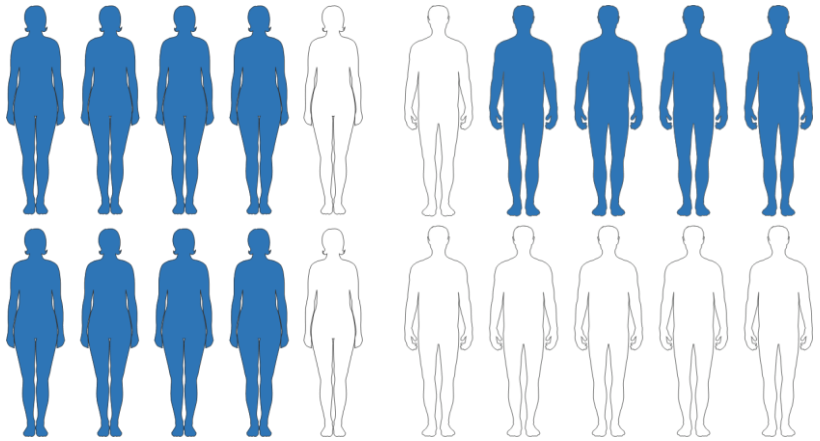
Characterised by e.g.

- Loss of control over intake,
- Drinking despite negative consequences (“compulsivity”)
- Relapse to alcohol abuse even after prolonged absitnence

WHO 2018

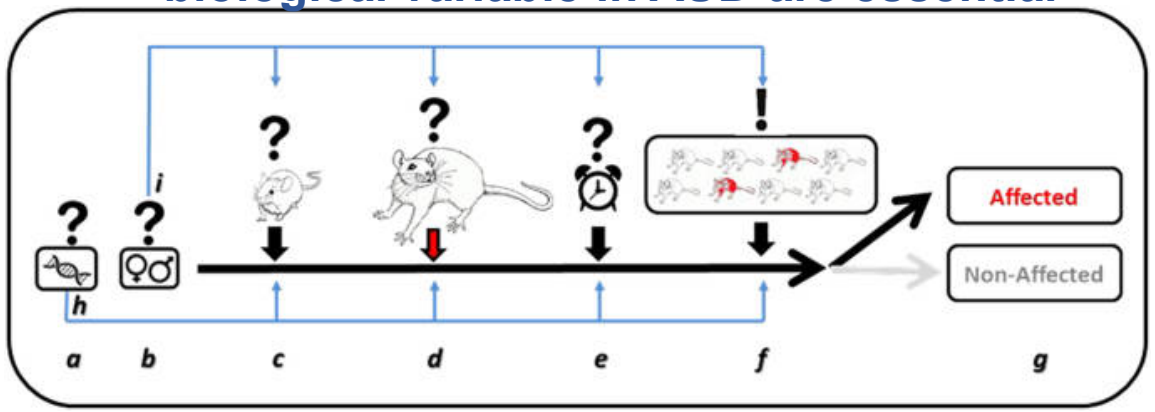
Sex differences in Alcohol Use Disorders

- While the prevalence of AUD higher in men – the gender gap is narrowing over time (*White et al., 2015*).
- Women more vulnerable to alcohol-induced pathophysiology (alcohol-induced liver inflammation and cardiovascular diseases (*White et al., 2020*).

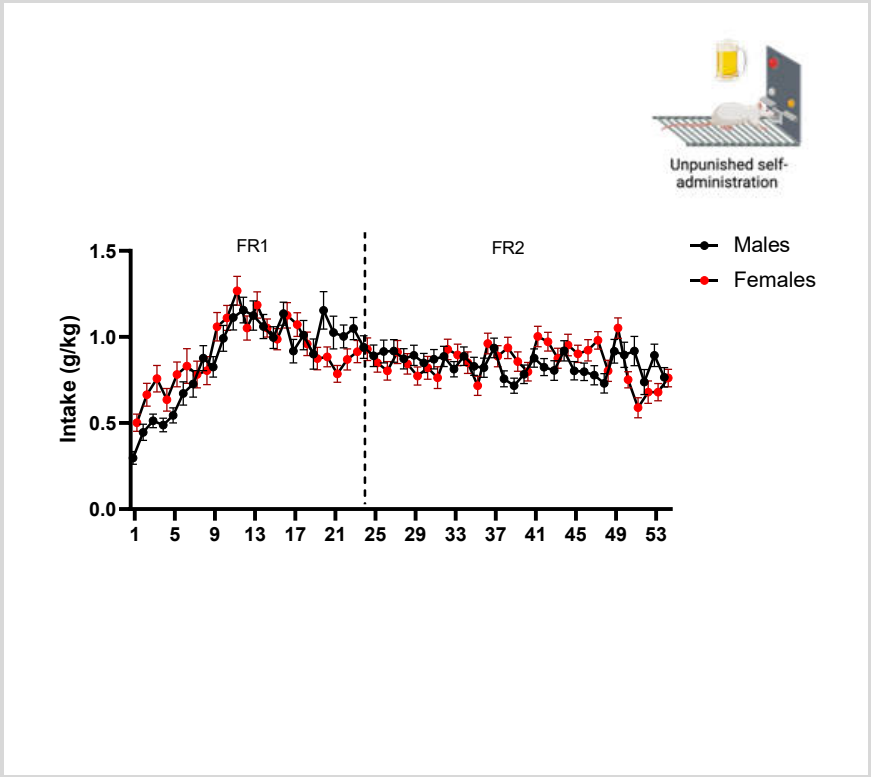


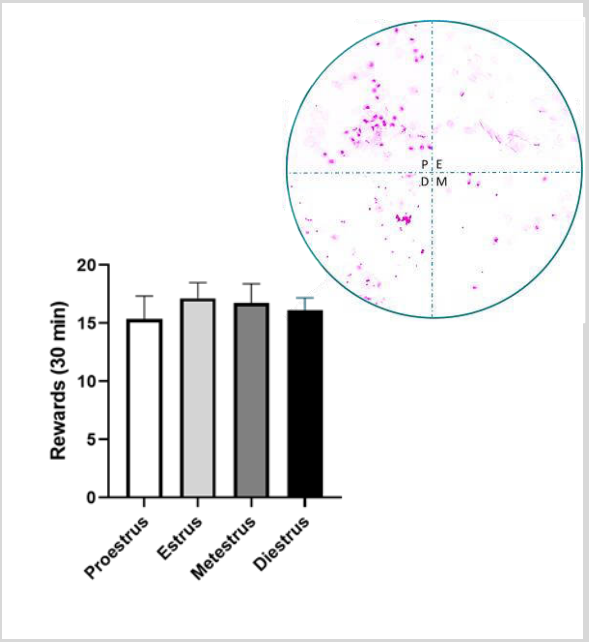
McLean et al., 2011; Bangasser et al., 2014

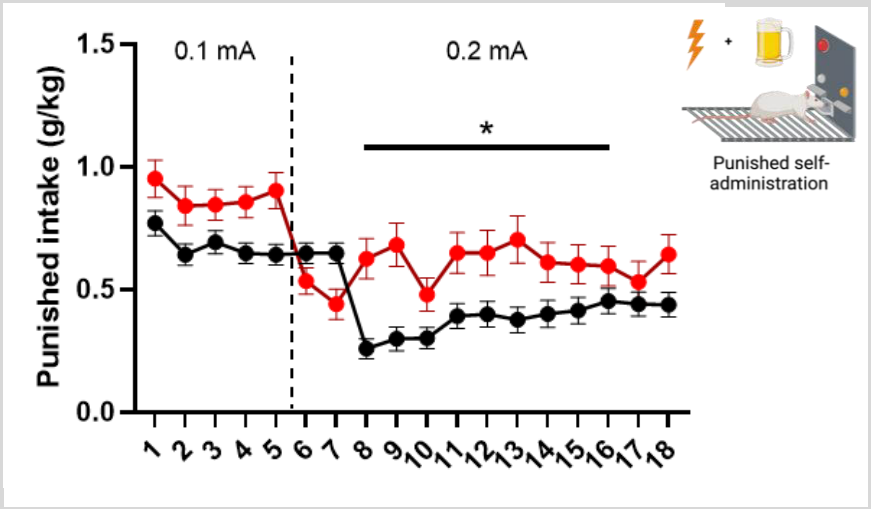
Preclinical models considering sex as a biological variable in AUD are essential



Sex differences in Alcohol Use Disorders



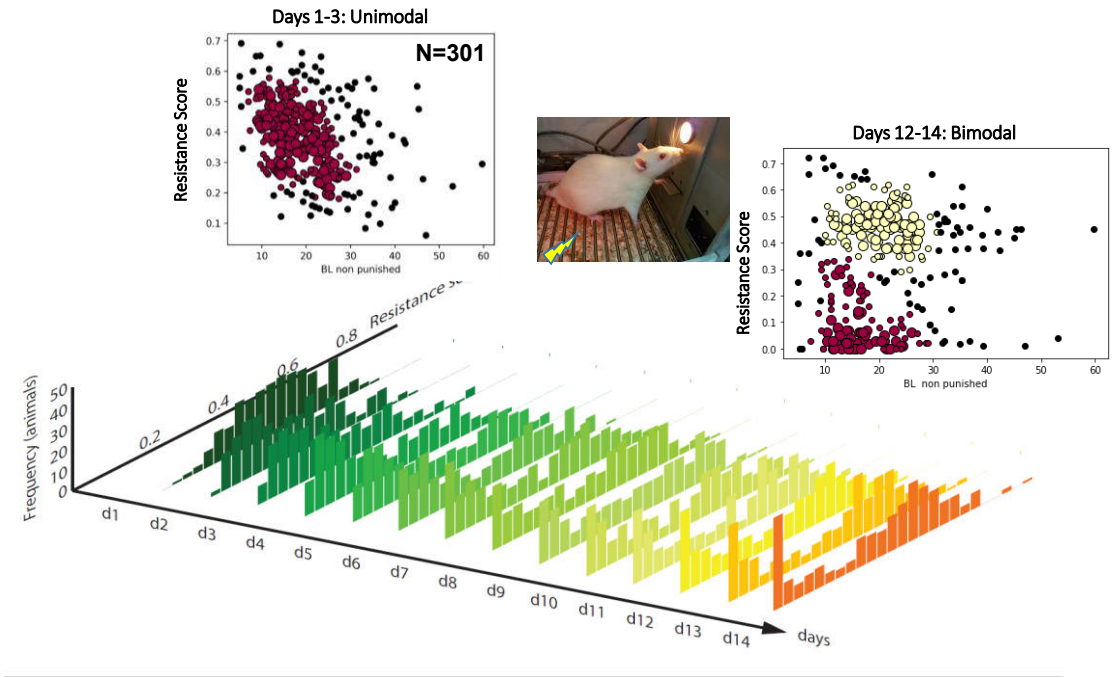




- Female alcoholics more likely to cite **negative emotions** and **stressful life experiences** as reasons for alcohol use and relapse (*Erol & Karpyak, 2015*)

Unpublished data

Sex differences in compulsive alcohol use



Domi et al.,Science Advances 2021

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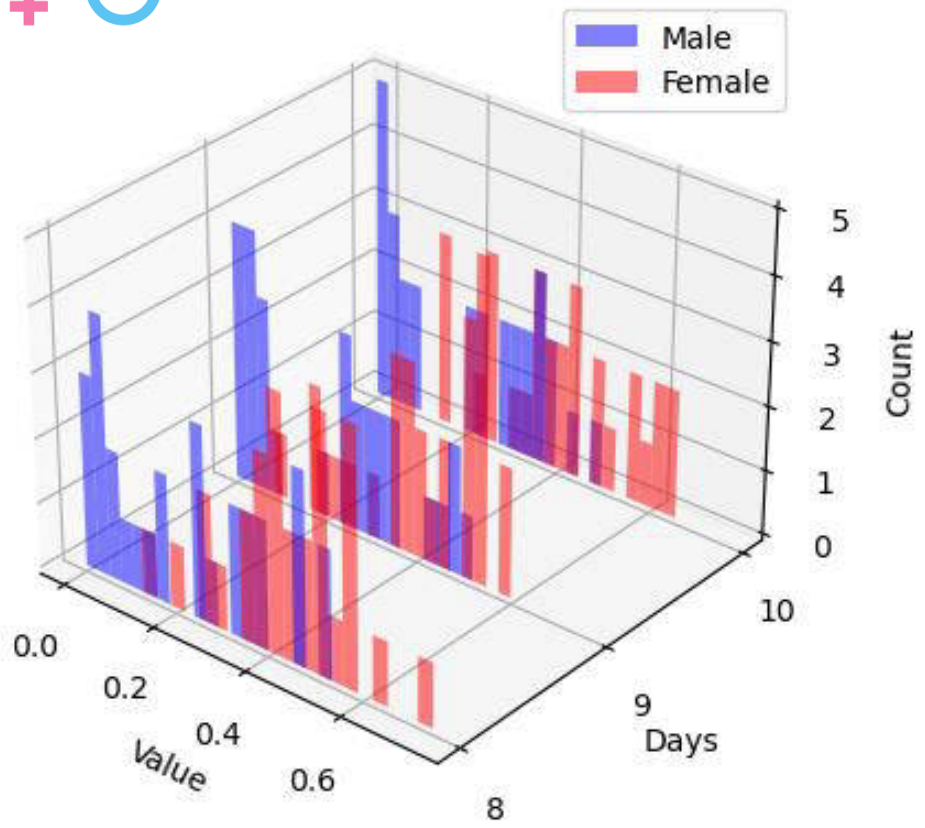
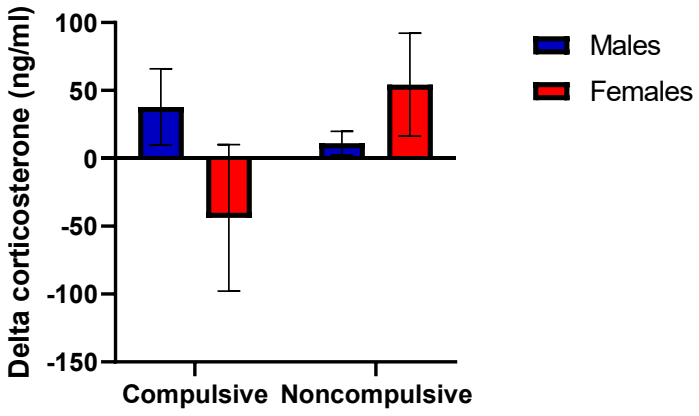
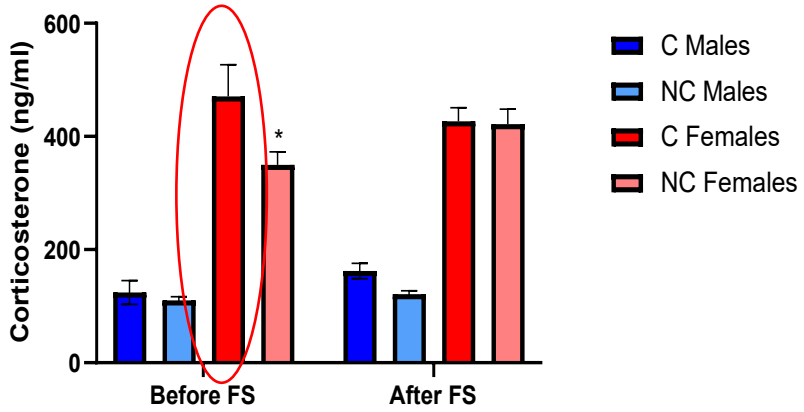
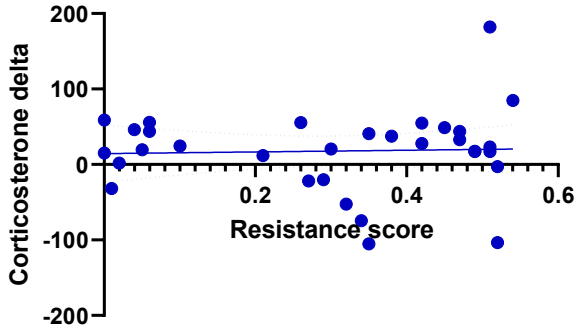


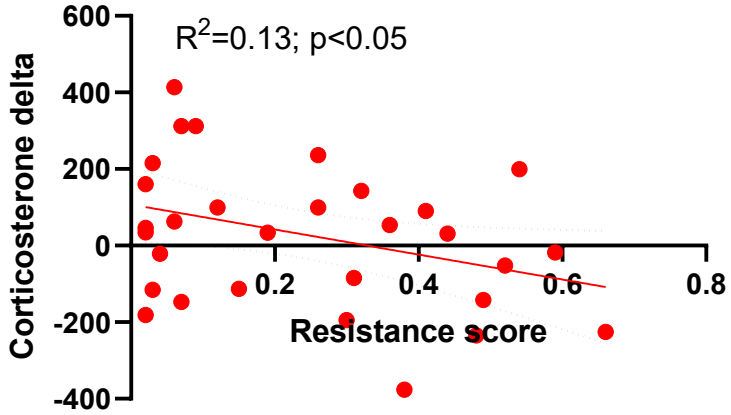
Unpublished data

Corticosterone as a predictive biomarker of compulsive alcohol use in female rats?









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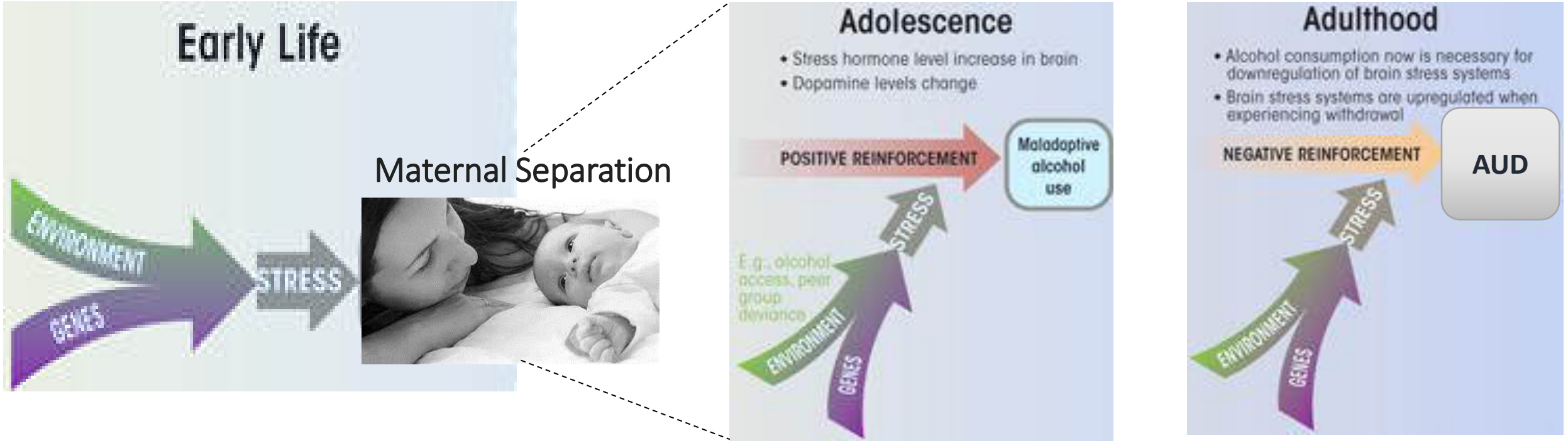
Early life stress important environmental risk factor for the development of AUD

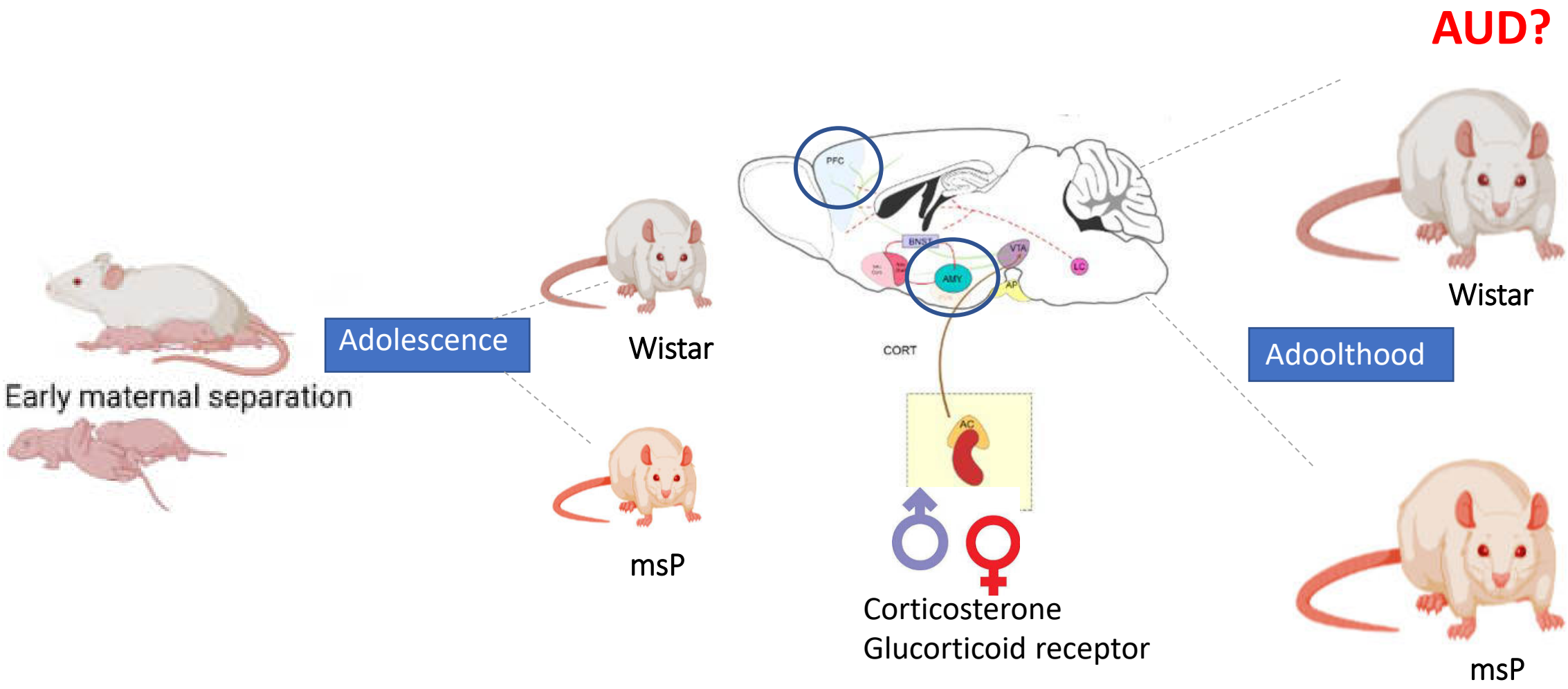


- 26% of adults, that has experienced stress before age 18, developed substance use disorders *(Green et al., 2010).*
- The effect of early life stress and childhood maltreatment is greater in women who are more susceptible to develop AUD in adulthood *(Anda et al., 2002; Dinwiddie et al., 2000).*

EARLY LIFE SOCIAL ISOLATION STRESS (ESI)

- **HPA axis disbalance** *(Nishi, 2020)*
- **Behavioral impairments: depressive-like or anxiety-like behaviors in adulthood** *(Huot et al., 2004; Menard et al., 2004)*
- **Neurochemical changes within the reward and stress pathway** *(Brake et al., 2004; Meaney et al., 2002)*
- **Psychiatric disorders, including AUD** *(Roman and Nylander, 2005*

Evaluate the effects of ESI on stress-induced neuroadaptations and vulnerability to develop AUD in Wistar and genetically selected msP rat lines



The msP rat line: a genetic model of predisposition to AUD

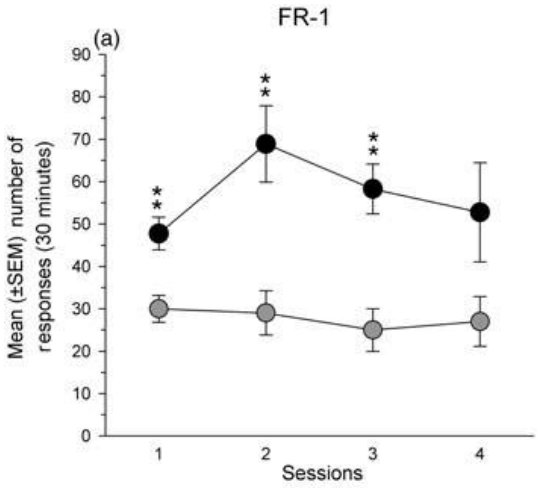
Wistar

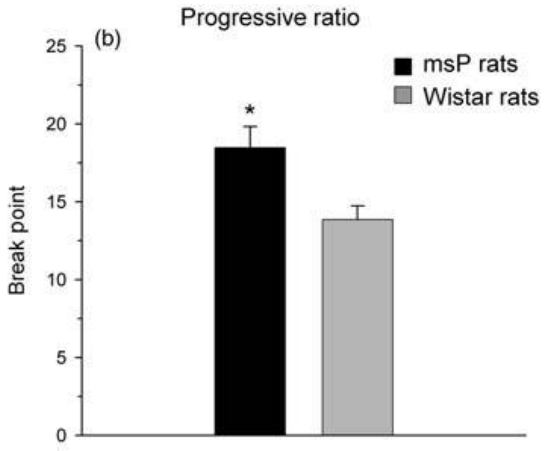
Selection for alcohol preference in Cagliari

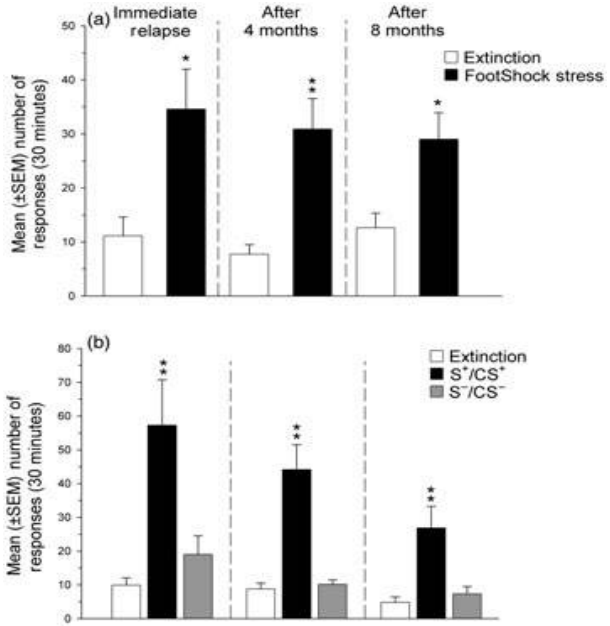
Sardinian alcohol preferring rats

Cohort to Camerino and further selection

Marchigian Sardinian alcohol preferring rats (msP)

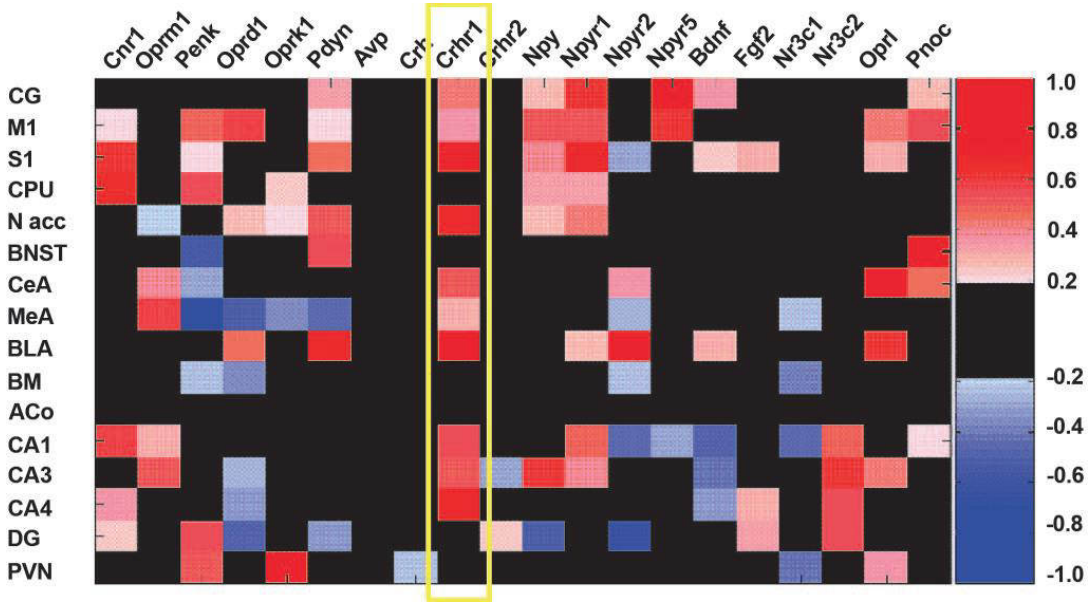


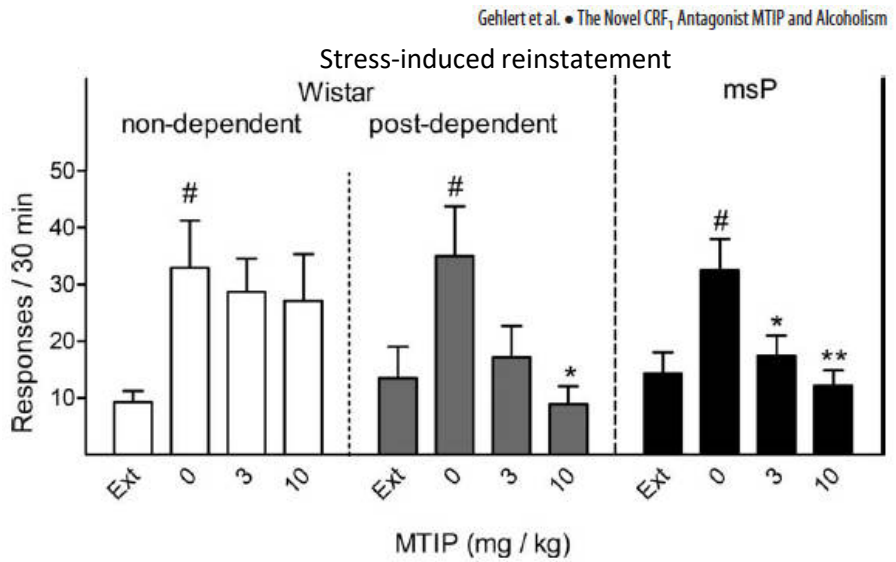


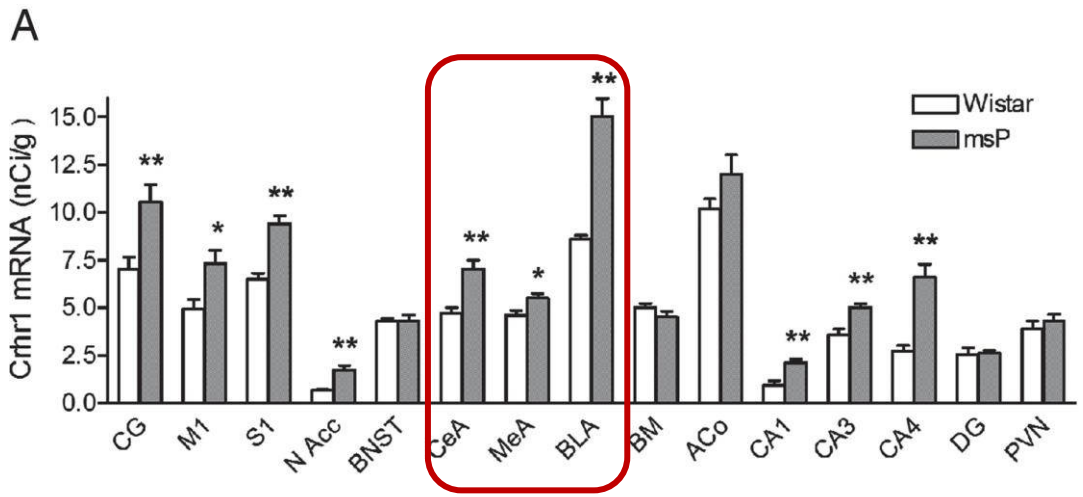


Ciccocioppo et al, Addiction Biology 2006

MsP rats carry a CRF1 gene variant inducing system overexpression







Hansson et al, PNAS 2006

msP

Enhanced:

- ✔ Anxiety like behavior
- ✔ Stress response
- ✔ Depressive like behavior
- ✔ AUD like behaviour

Experimental design

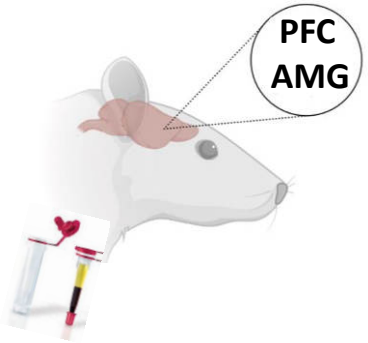
Male and female msP and Wistar rats

(Rice and Barone, 2000; Lister et al., 2013)

Plasma, Brain collection

PND0 PND14 PND21 PND35 PND75

AUD related behaviors

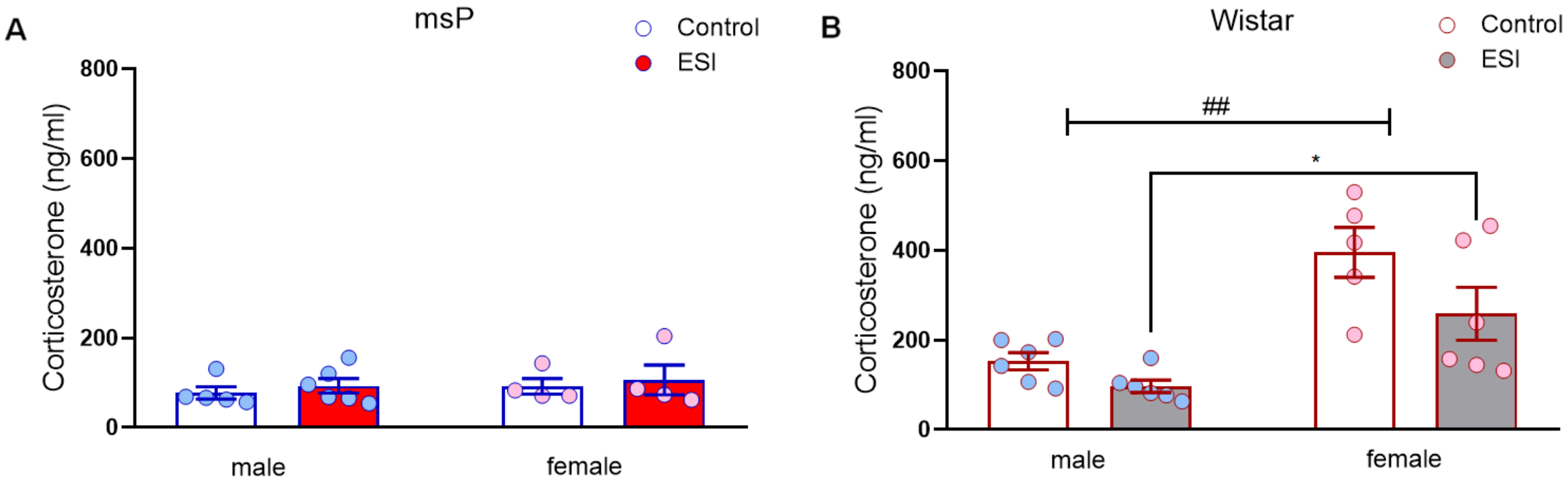


EARLY SOCIAL ISOLATION (ESI)

30min per day

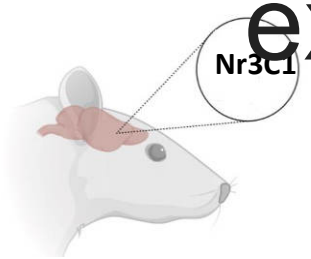
1 week

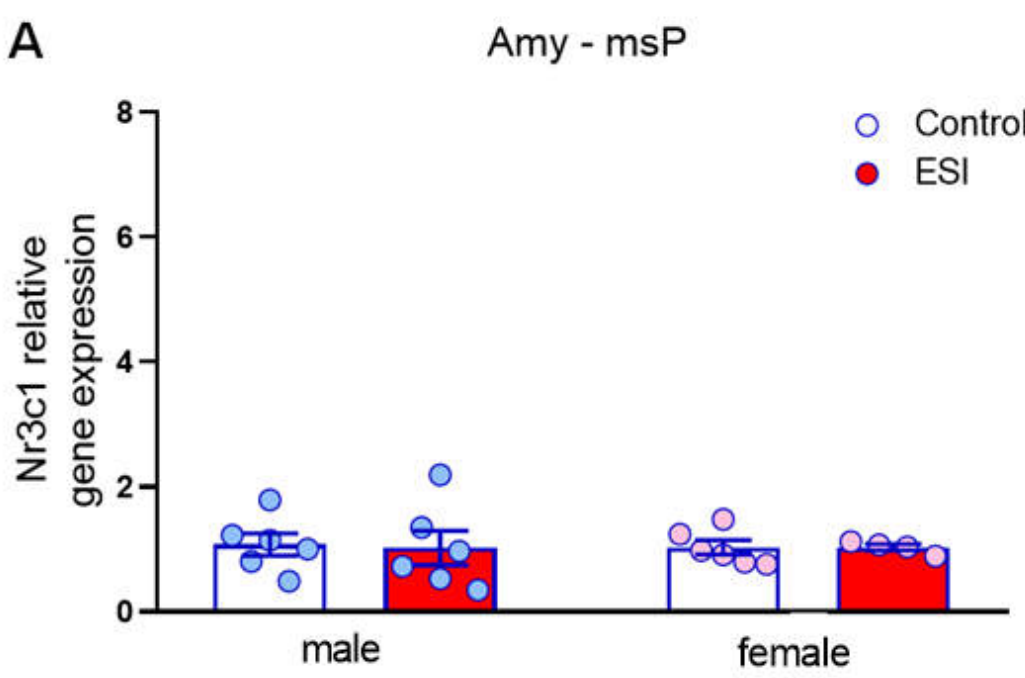
Female Wistar rats show higher corticosterone levels compared to male rats

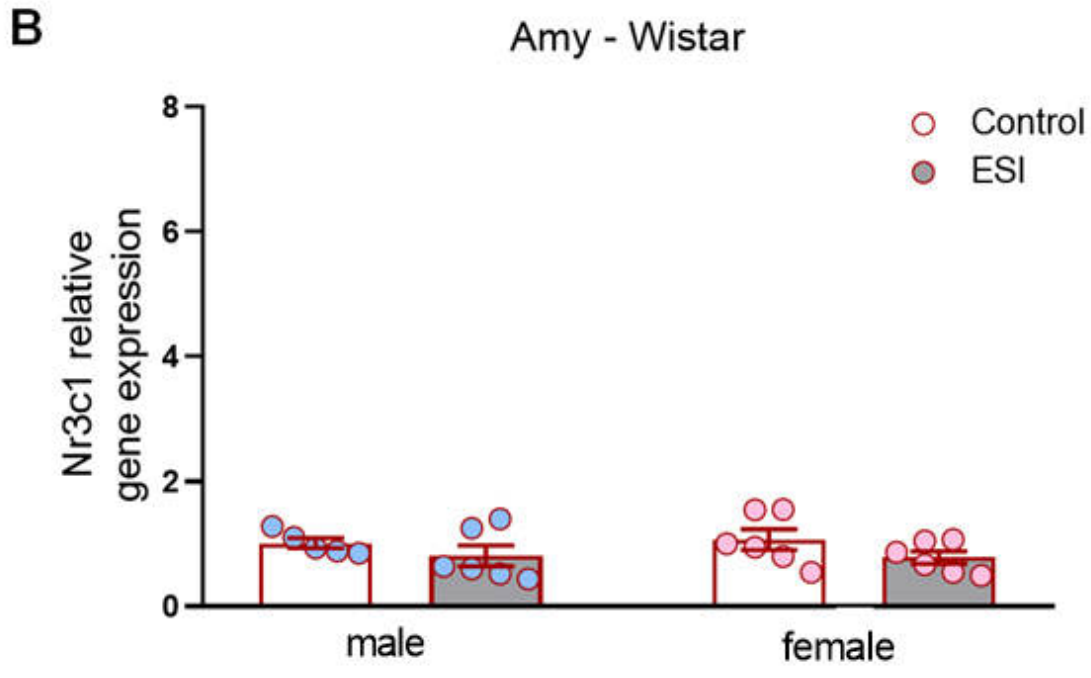


Unpublished data

Glucocorticoid receptor gene (NR3C1) expression was not altered in the amygdala

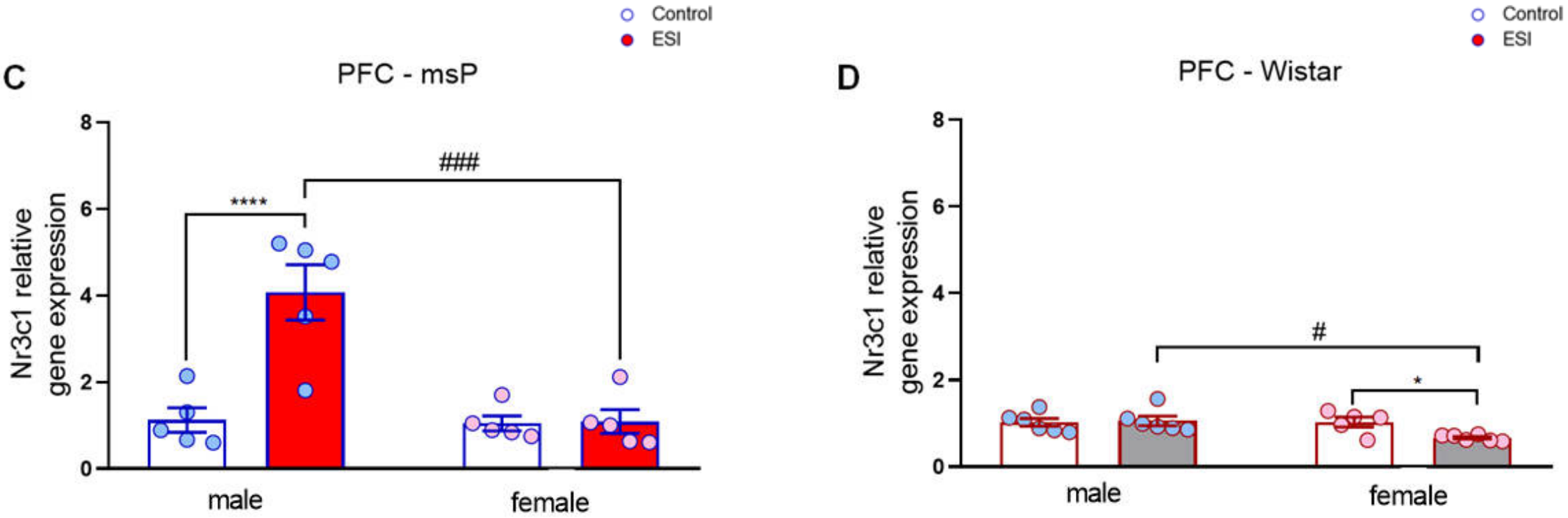






Unpublished data

Glucocorticoid receptor gene (NR3C1) was differentially expressed in the PFC of male and female msP and Wistar rats exposed to ESI



Unpublished data

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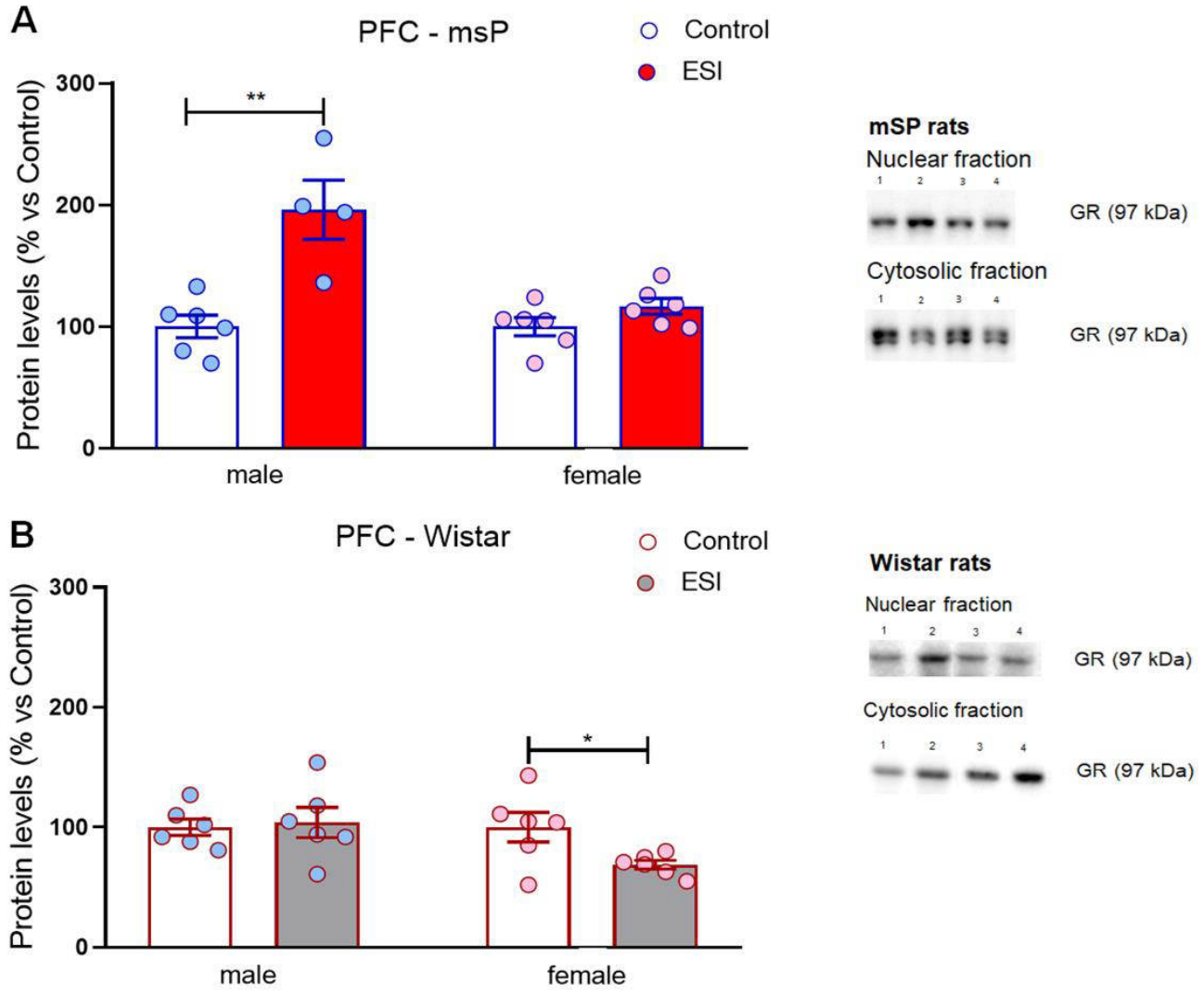
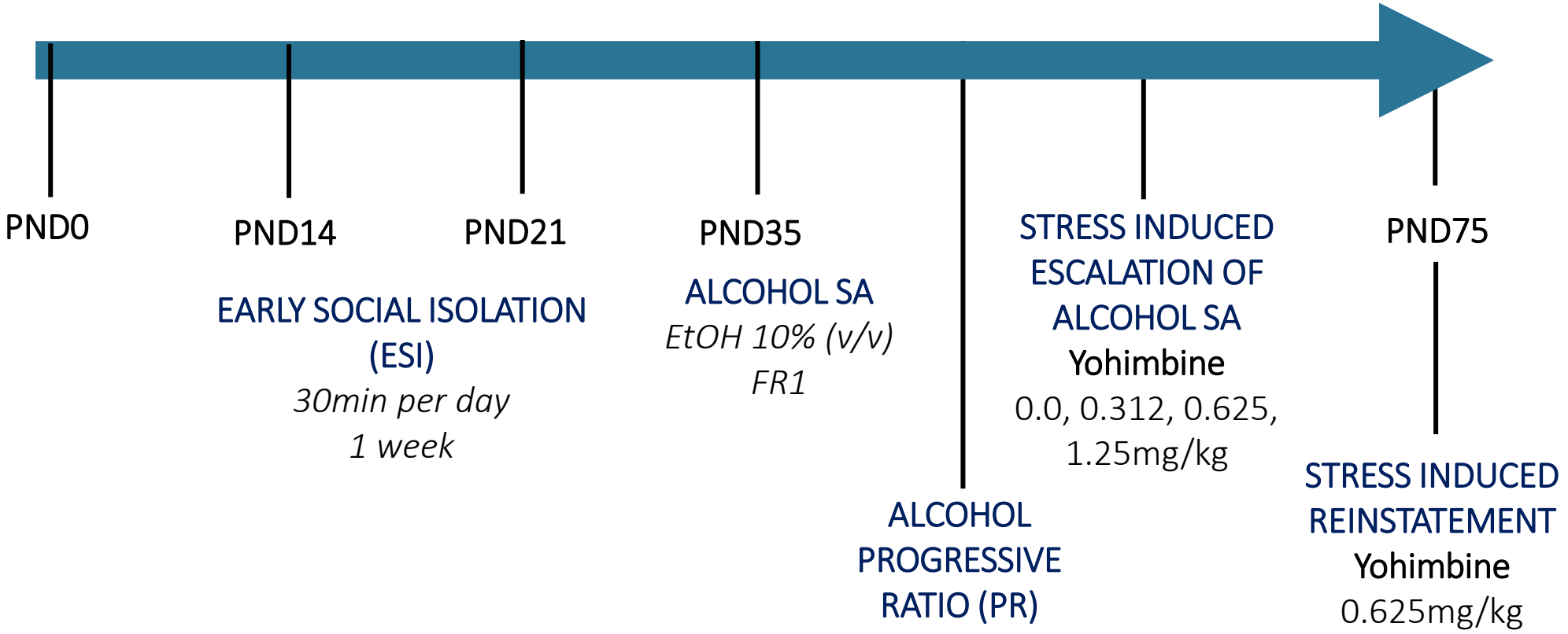


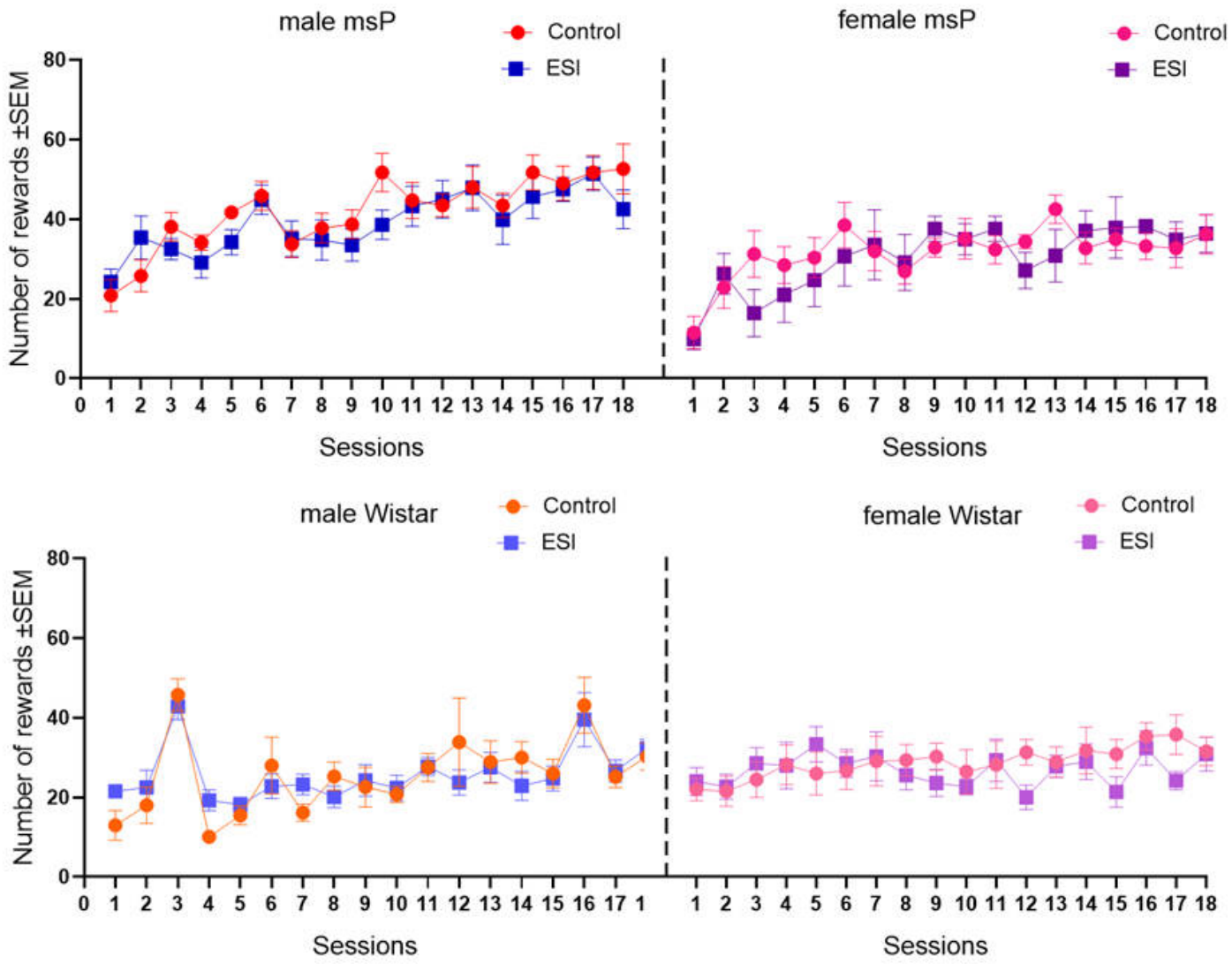
Unpublished data

The effects of maternal separation on AUD related behaviours

Male and female msP and Wistar rats

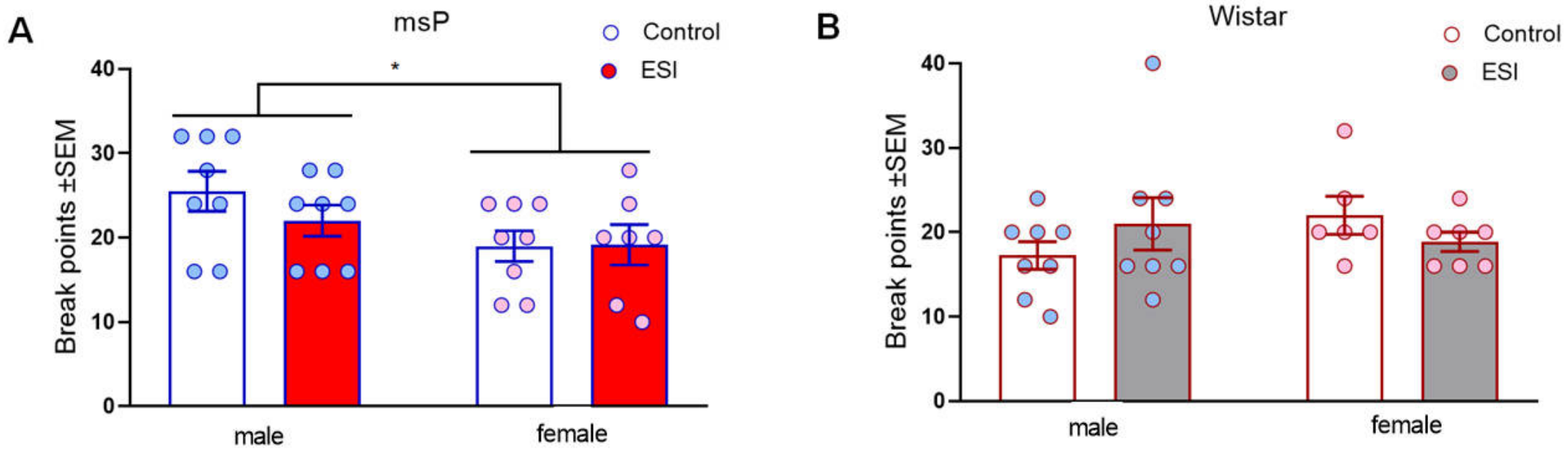


Alcohol drinking was not affected by early life social isolation



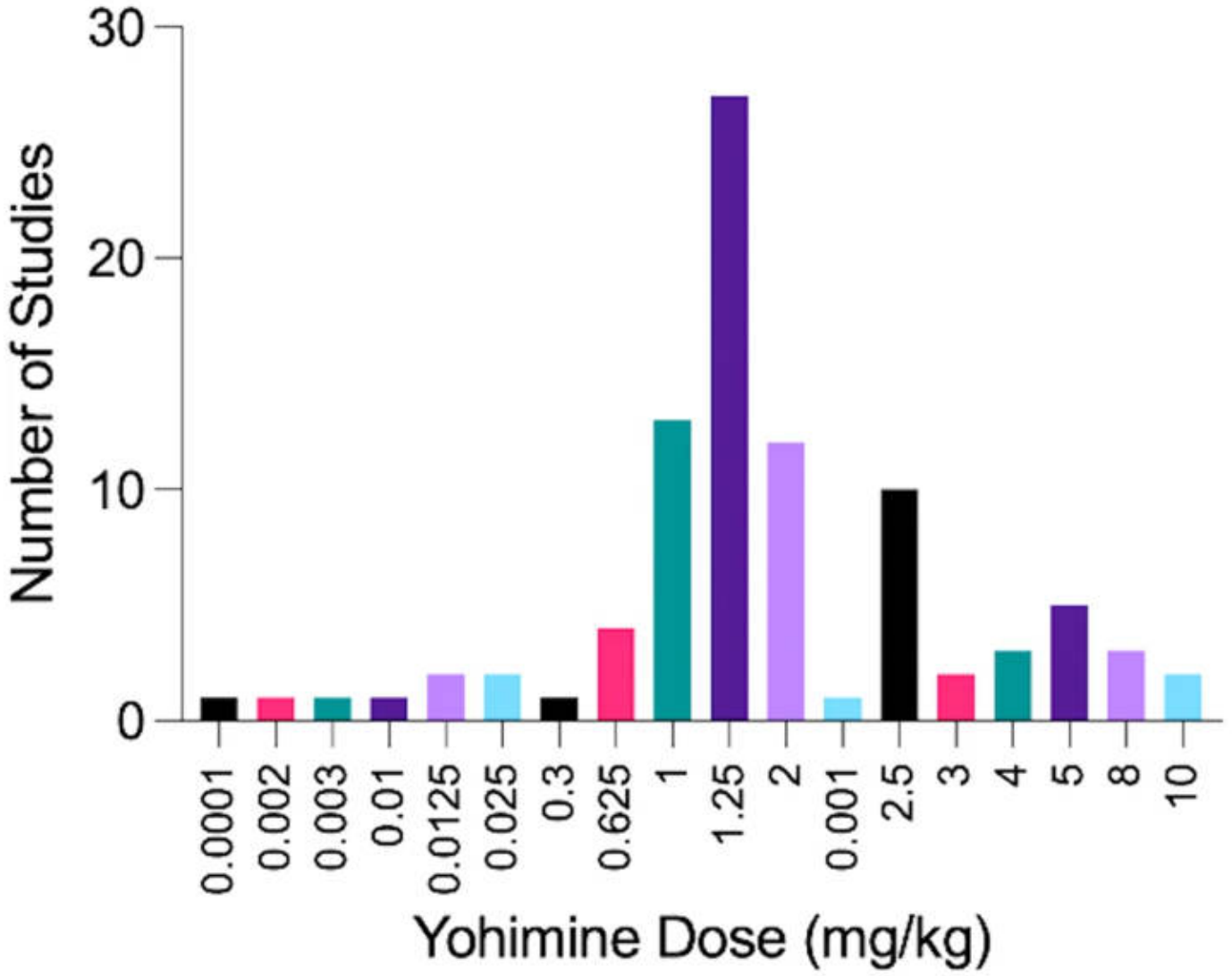
Unpublished data

msP male rats showed a higher motivation to drink alcohol compared to female rats



Unpublished data

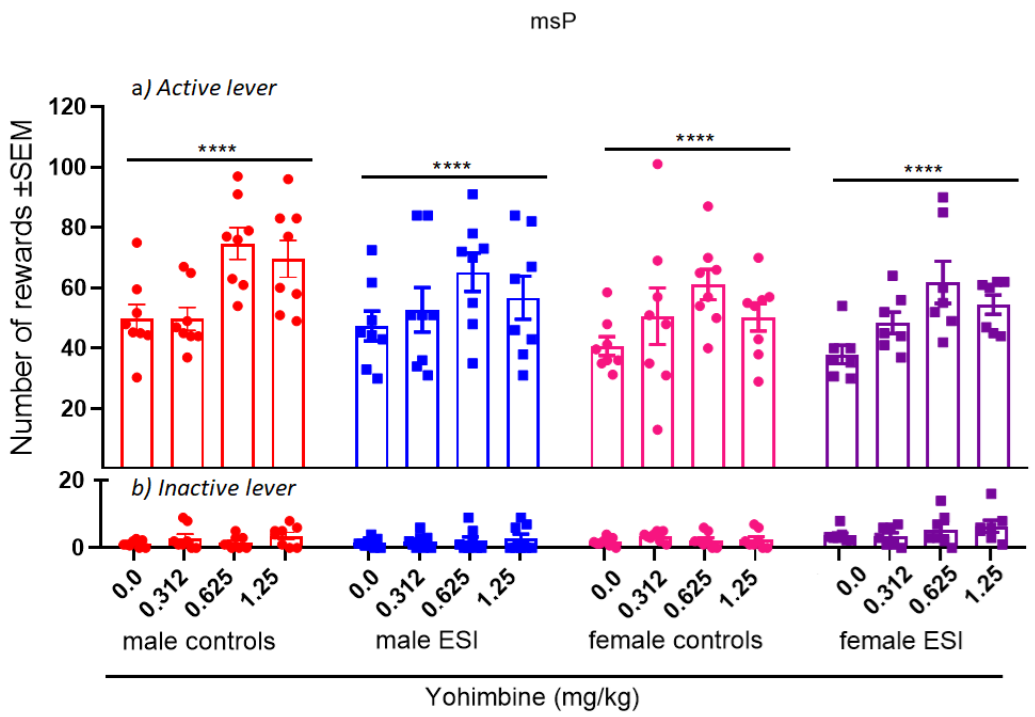
Yohimbine as a pharmacological probe for alcohol research in rodents and humans

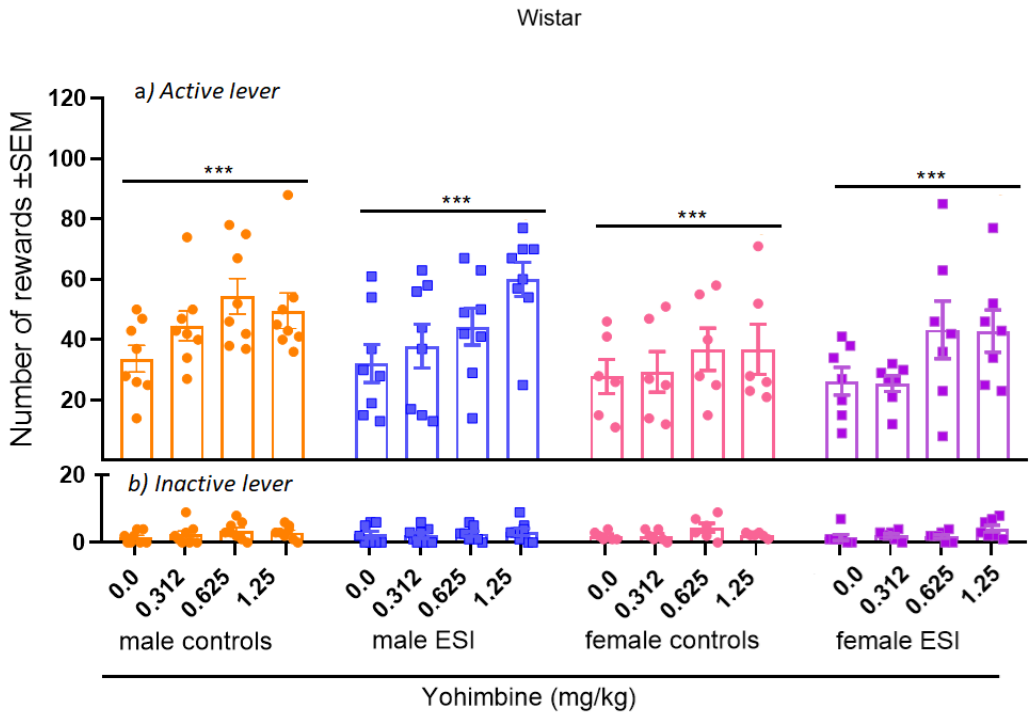


Major limitations include: the lack of inclusion of sex as a biological variable

Curley… Ciccocioppo, Koffler 2022

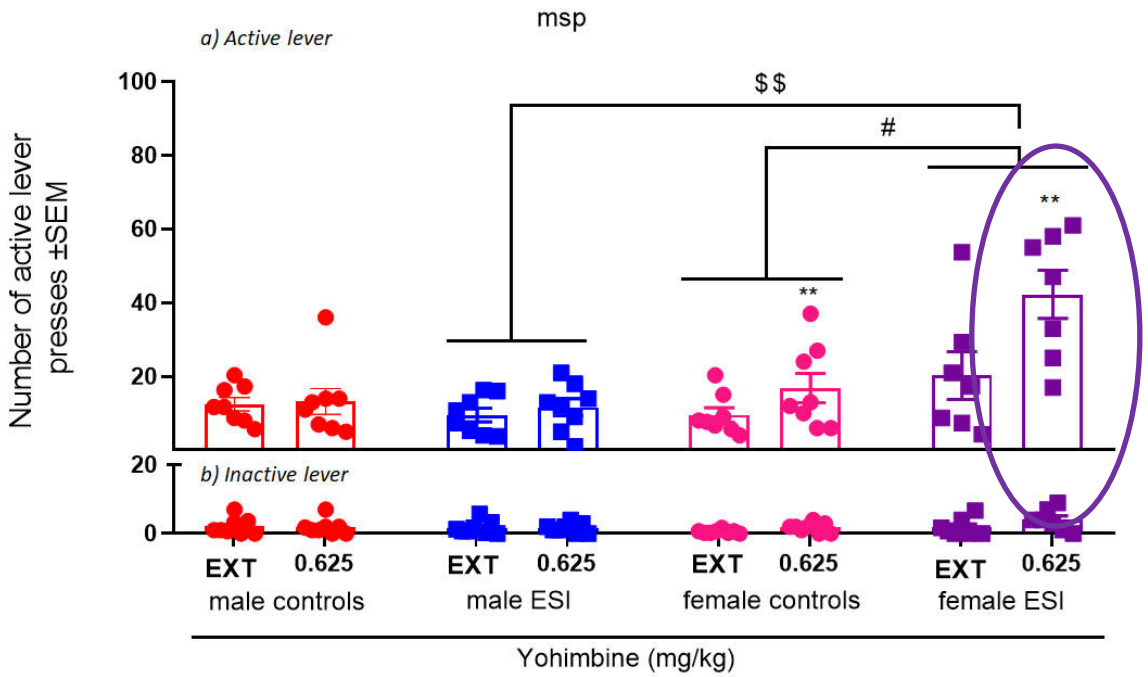
Yohimbine increased alcohol intake in both male and female msP and Wistar rats

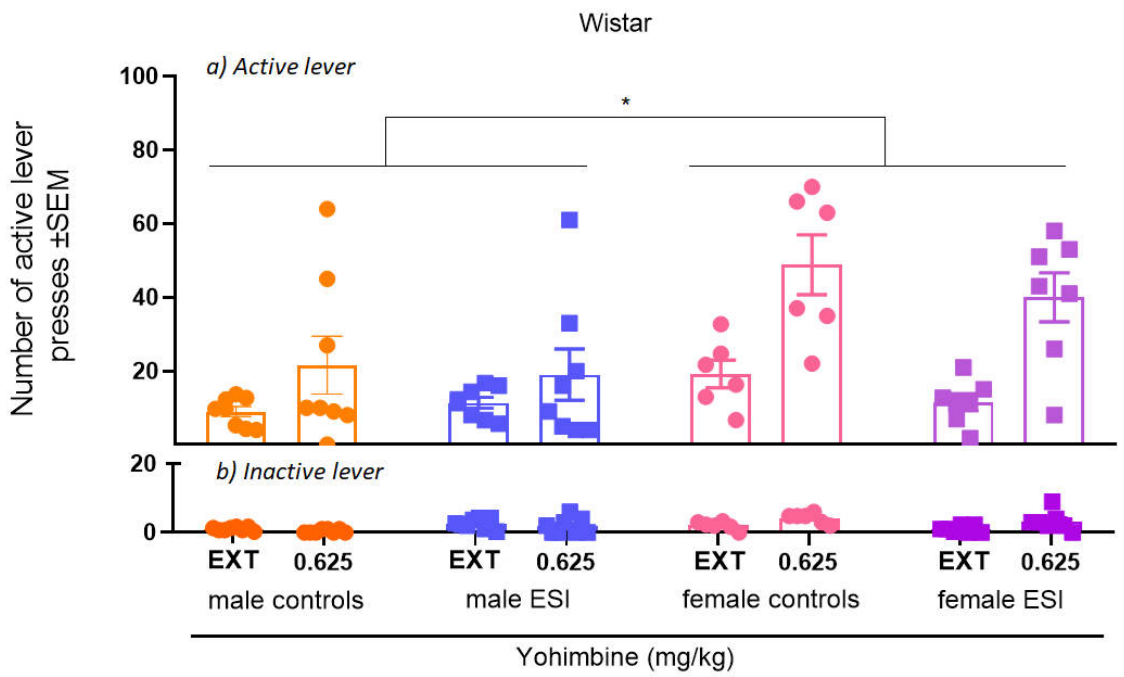




Unpublished data

msP female rats previously exposed to maternal separation showed a higher yohimbine induced relapse to alcohol seeking

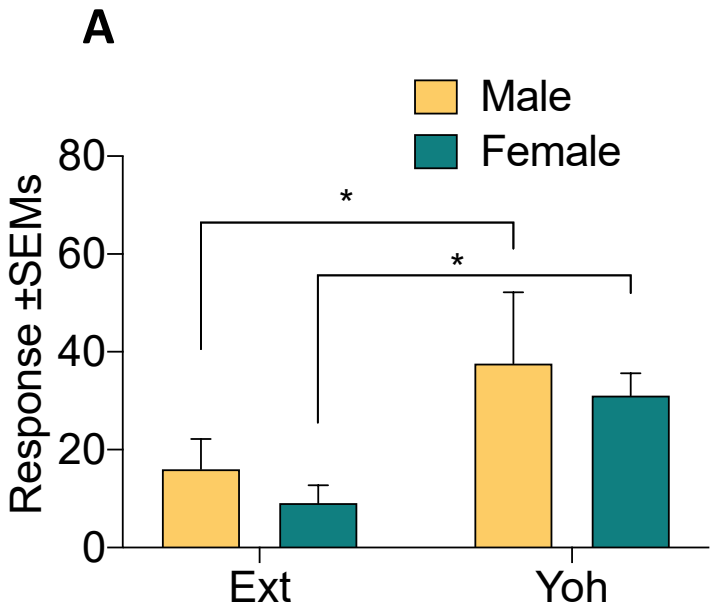




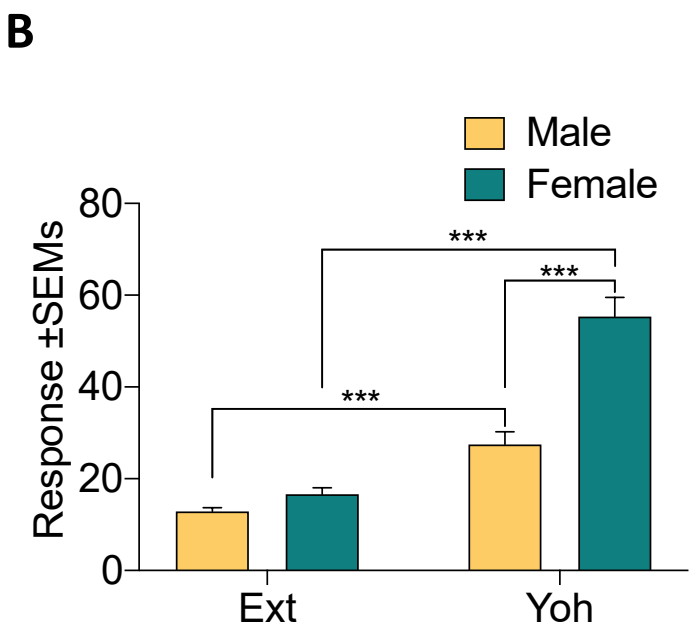
Unpublished data

Yohimbine exacerbated cued reinstatement of alcohol seeking in female

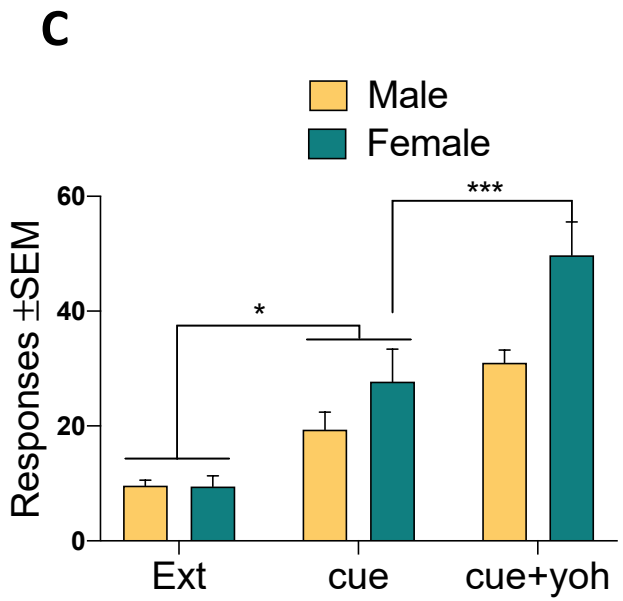
Wistar rats



msP rats



msP rats



Cannella, unpublished data

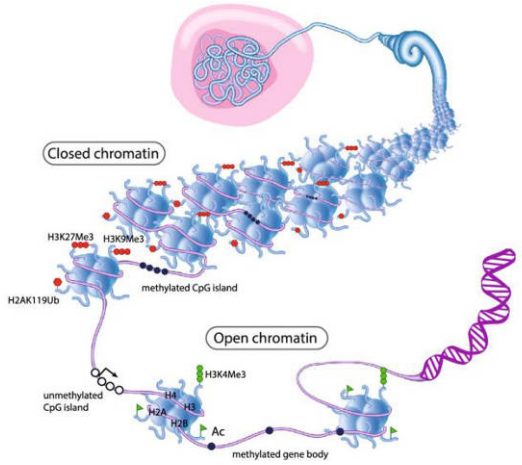
We found sex as a biological variable to the effects of maternal separation on glucocorticoid receptor neuroadaptations and to the effcects of yohimbine in relapse to alcohol seeking behaviour

Early Life Stress

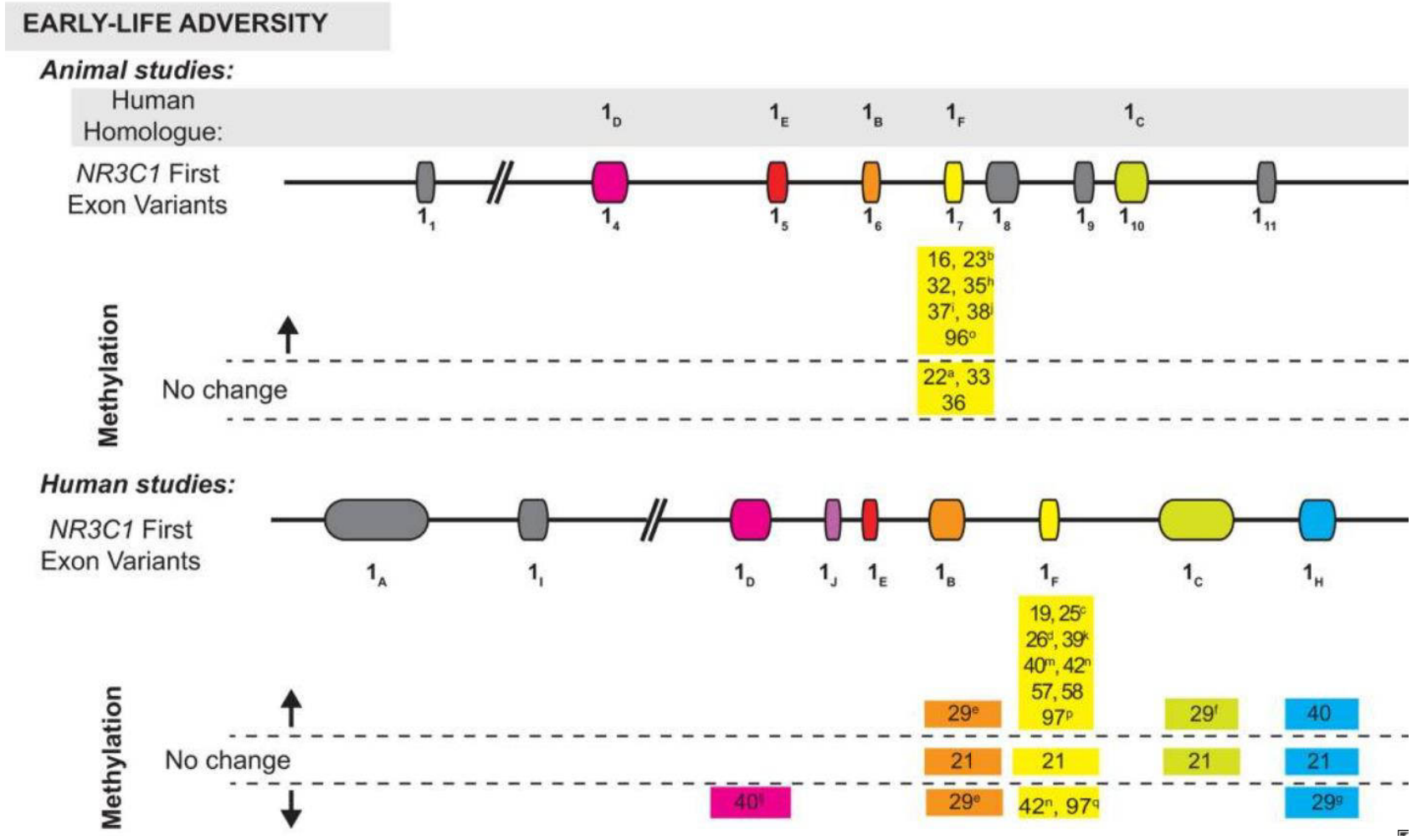
Enhanced:

- ✔ Stress response
- ✔ Downregulation of NR3C1
- ✔ AUD like behaviour

Epigenetics?



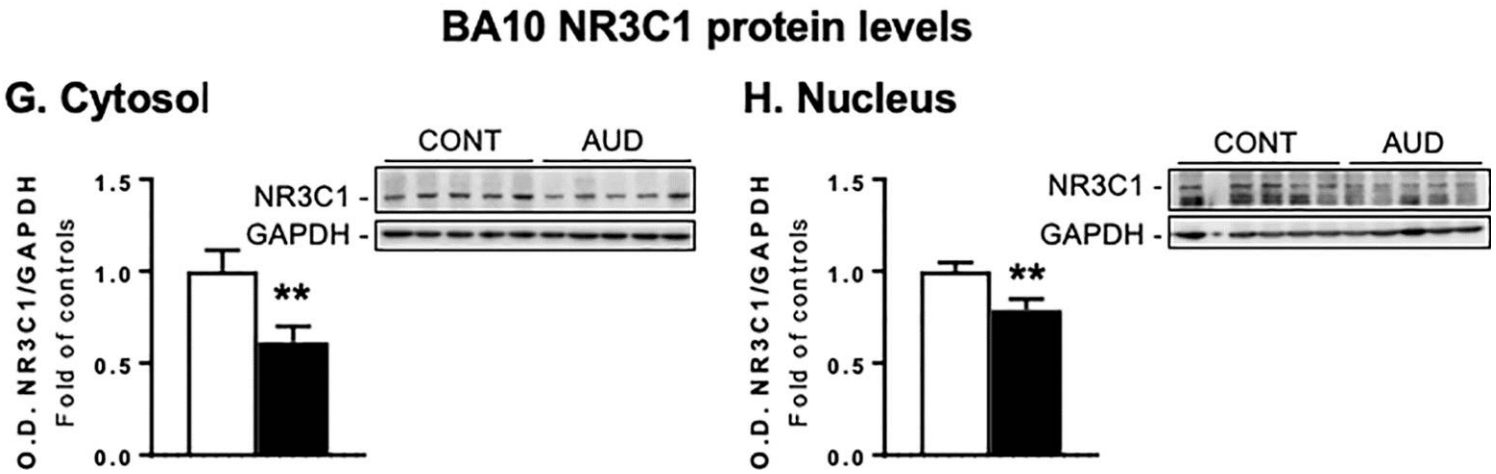
Early-life adversity reported increased methylation at GR at the exon variant 1F in both humans and animal studis



Turecki and Meaney, Biol Psychiatry 2016

NR3C1 expression is downregulated in the PFC of AUD patients

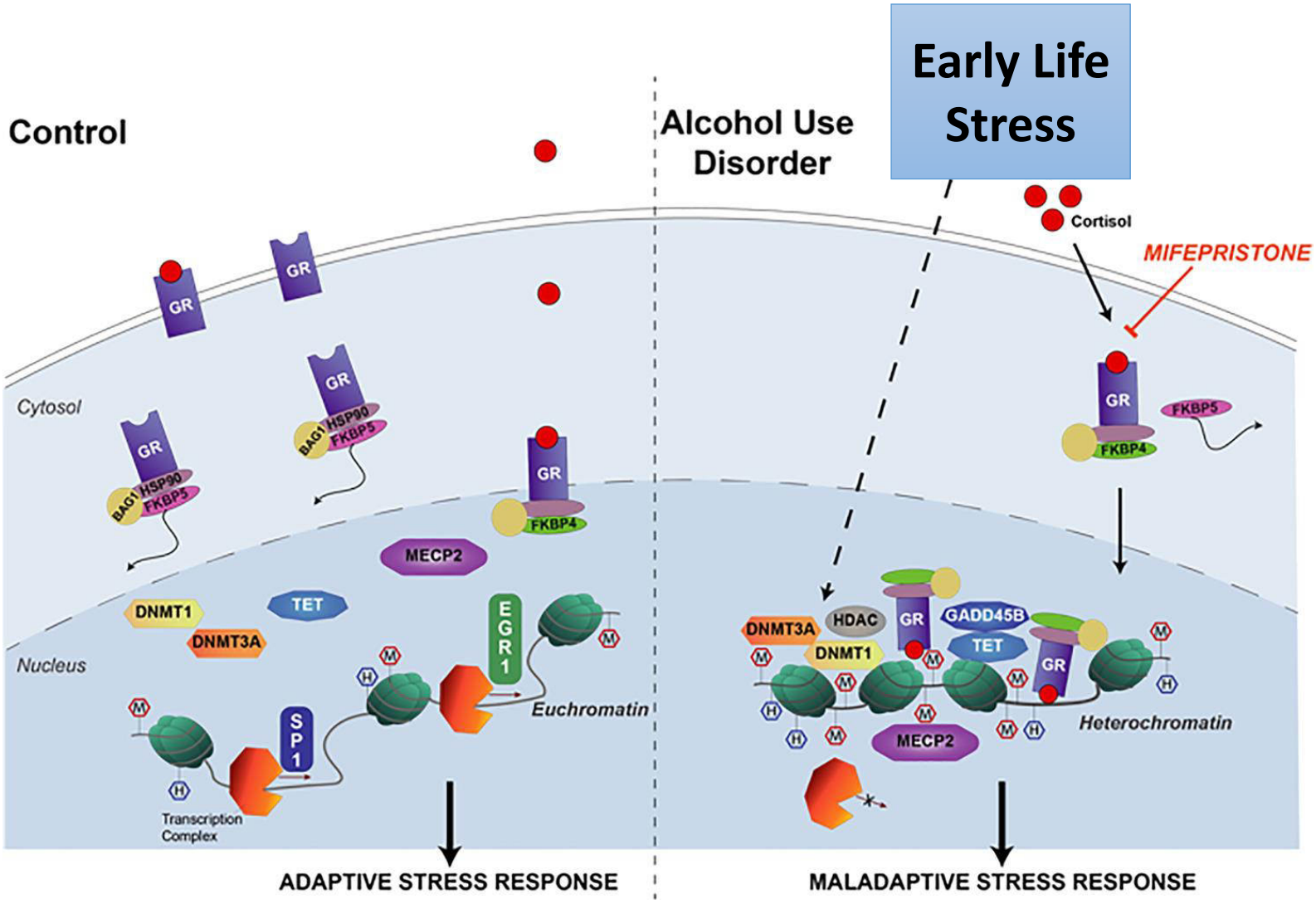
NR3C1 may play a crucial role in the pathophysiology of AUD.

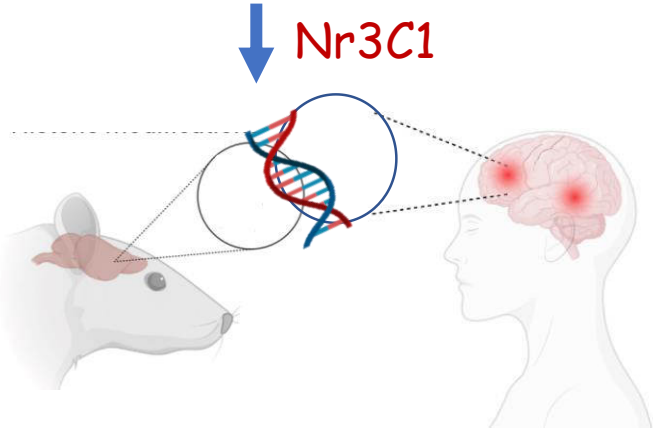


Gatta et al., Mol Psychiatry 2019

New South Wales Brain Tissue Resource Centre (NSW BTRC, University of Sydney, Australia)

Stress response via an epigenetic downregulation of Nr3C1 and Increased risk to develop AUD





Increased Risk to develop AUD

Thank you

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Prof. Nazzareno Cannella
Prof. Massimo Ubaldi
Dott.ssa Laura Soverchia
Sara de Carlo

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Prof.ssa Liana Fattore
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Prof.ssa Patrizia Romualdi
Prof. Fabio Fumagalli

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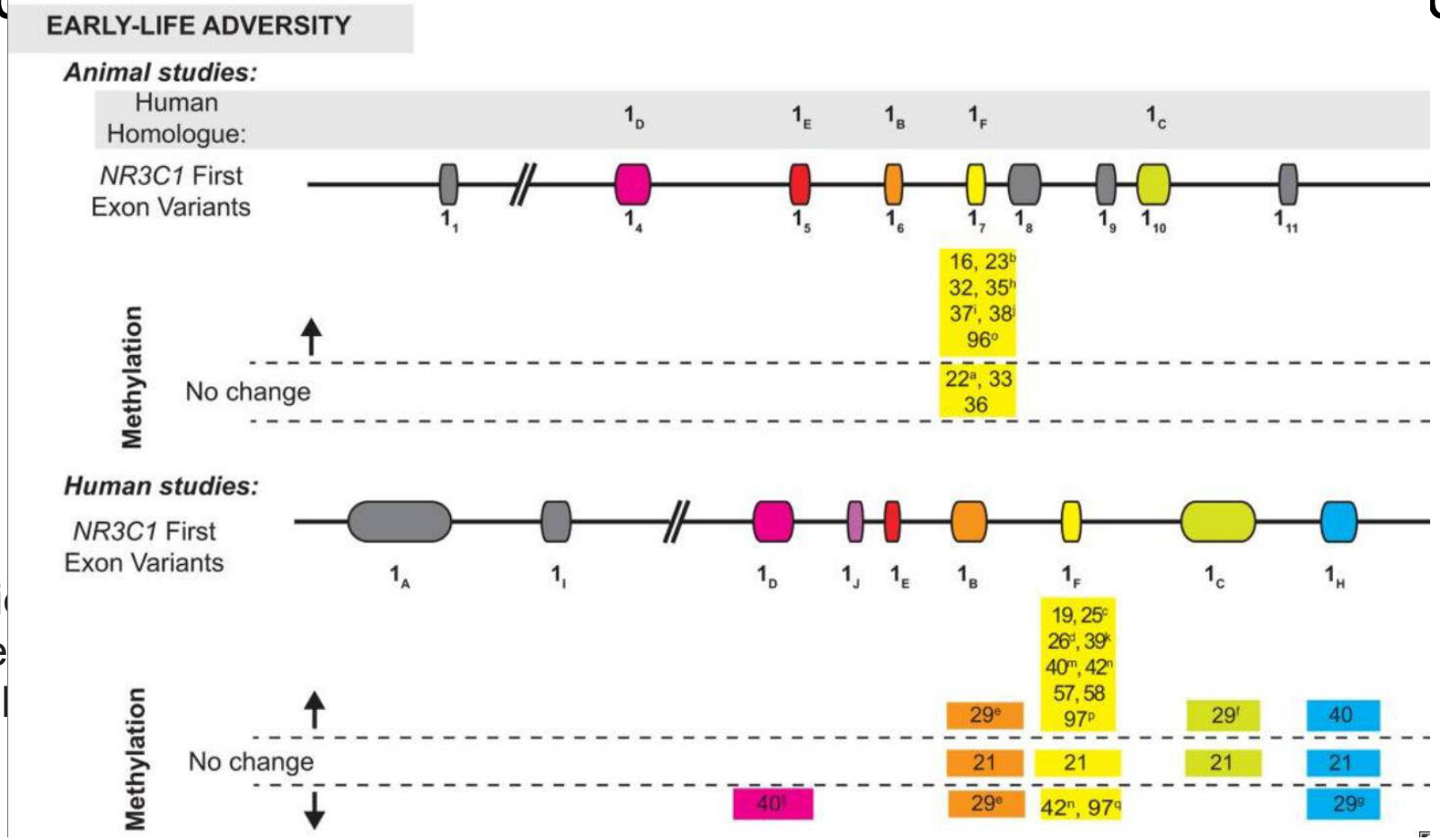
Genome-wide methylation in alcohol use disorder subjects: implications for an epigenetic regulation of the cortico-limbic glucocorticoid receptors (NR3C1)

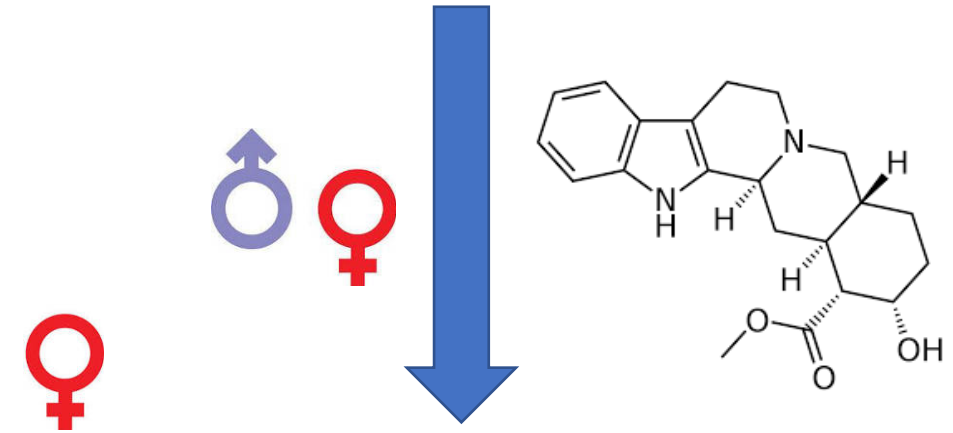


89% of human studi
early-life adversity re
at the exon variant 1

New South Wales Brain Tissue Resource Centre (NSW BTRC, University of Sydney, Australia)

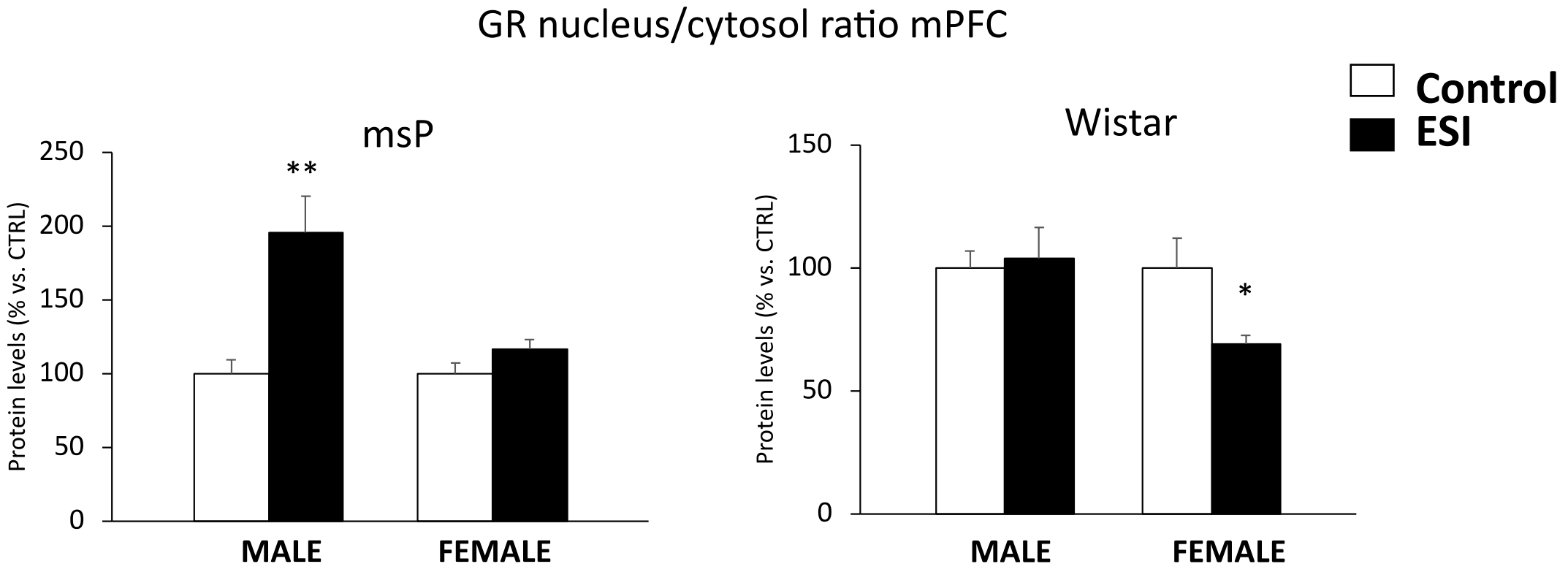
Summary and Future prespectives

We found sex as a biological variable to the effects of maternal separation and yohimbine in relapse to alcohol seeking behaviour

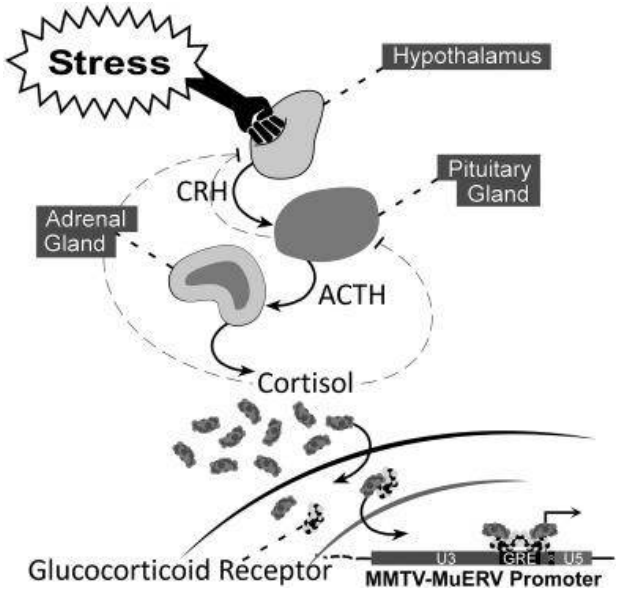


Yohimbine as a translational pharmacological tool to study stress-induced alcohol and anxiety like behaviours in humans

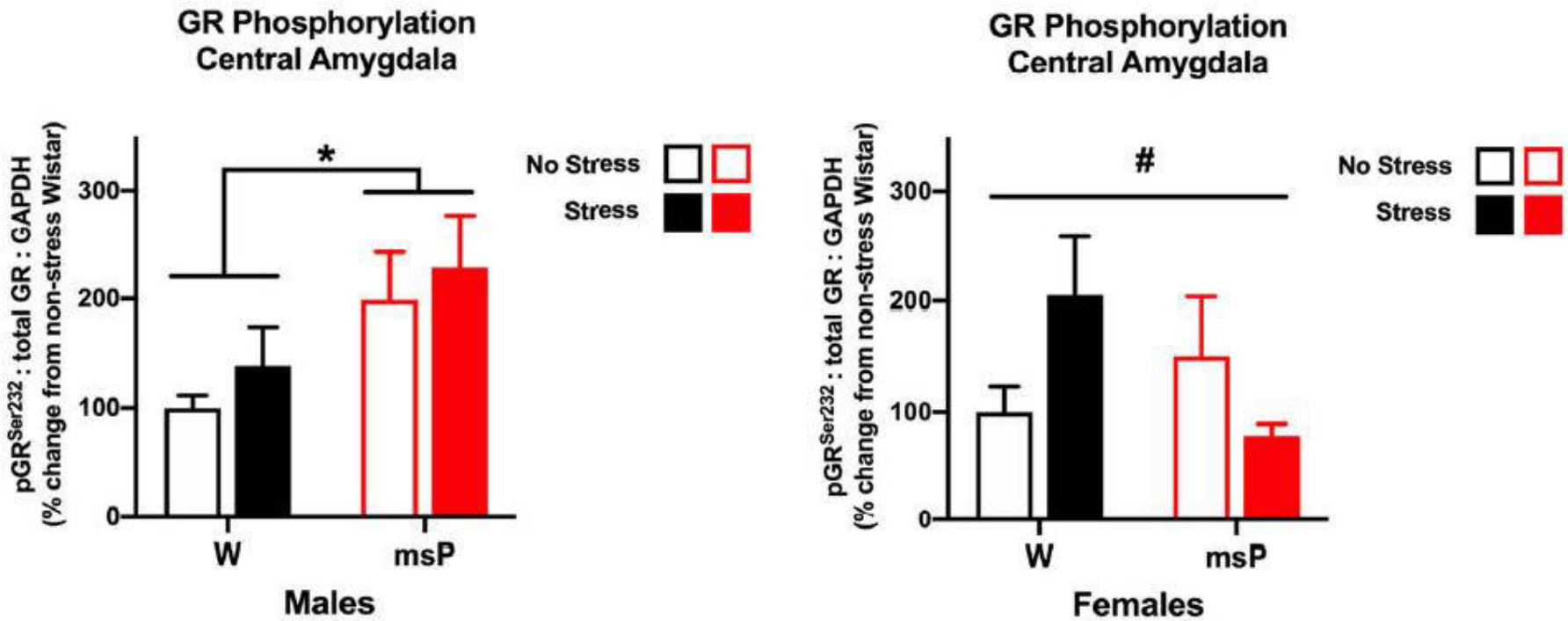
Glucocorticoid receptor expression was differentially expressed in the PFC of male and female msP and Wistar rats exposed to ESI



Unpublished data



Those results suggest that GC blockade may represent a promising pharmacotherapeutic approach for preventing future alcohol exposures [44]



(Natividad et al., 2021)

subsequent preclinical studies have shown that mifepristone in genetically selected msP rats was less efficacious in reducing alcohol consumption compared to Wistar rats [102]. The reduced effect of mifepristone in msP rats in alcohol-related behaviors are consistent with our clinical findings, where mifepristone significantly reduced craving only in patients with *low* FHDA. Therefore, mifepristone is less likely to be effective in patients with more genetically-based AUD [102].

Considering the consistent mifepristone suppression of alcohol craving and urge during the cue reactivity procedure, and the potential confounding by placebo effect on alcohol consumption discussed above, it is possible to speculate that mifepristone administration may be effective only in subjects with *low* stress at baseline. This potential explanation is consistent with previous preclinical studies showing that mifepristone suppresses alcohol drinking in Wistar, but not in msP rats [102].

Although stress [1, 2] has long been linked to alcohol intake and reoccurrence during abstinence, there are no available medications that specifically target stress-induced alcohol consumption. It

GCs, which interact with many neuroendocrine systems [30] and mediate adaptation to stress response through the HPA axis feedback action [31, 32], constitute another pharmacological target.

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In our original preclinical work, we demonstrated that, systemic and infusion of mifepristone in the central nucleus of the amygdala, reduced yohimbine-stress induced reinstatement of alcohol-seeking in alcohol-dependent rats [18]. In a rhesus macaque model, mifepristone also decreased daily alcohol self-administration [42]. The effect of mifepristone reducing alcohol seeking was also further supported by translational studies in alcohol-dependent rats and individuals with AUD [43].

Those results suggest that GC blockade may represent a promising pharmacotherapeutic approach for preventing future alcohol exposures [44]. However, in conditions without stress-induction, mifepristone has no effect on alcohol intake [18, 35, 45, 46], corroborating the hypothesis that GC receptors represent a key element in the preoccupation/anticipation (craving) stage of the addiction cycle. What remains unknown is the effect of mifepristone in acute stress precipitating alcohol craving and consumption in humans.

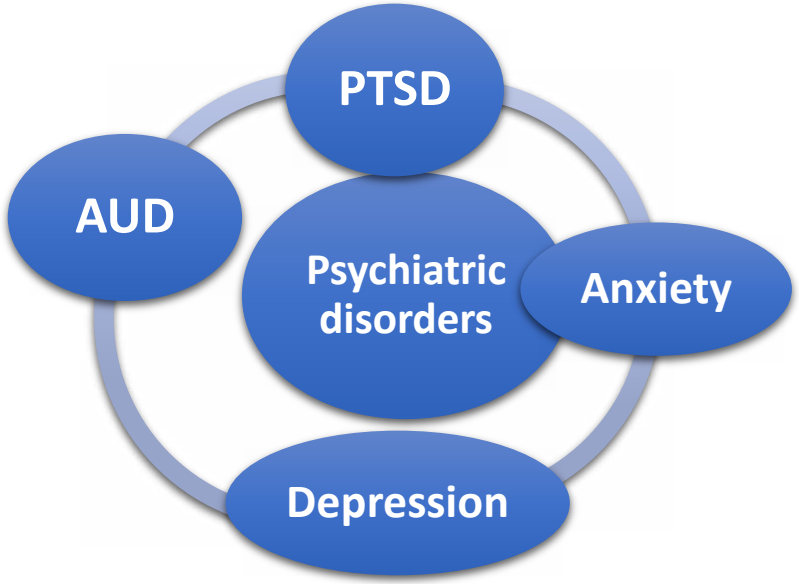
Stress is an important environmental risk factor for the development of AUD

Trauma

Social stress

Witness stress

Dysregulation of the CRF system and HPA axis



Stress is an important environmental risk factor for the development of AUD

Trauma

Social stress

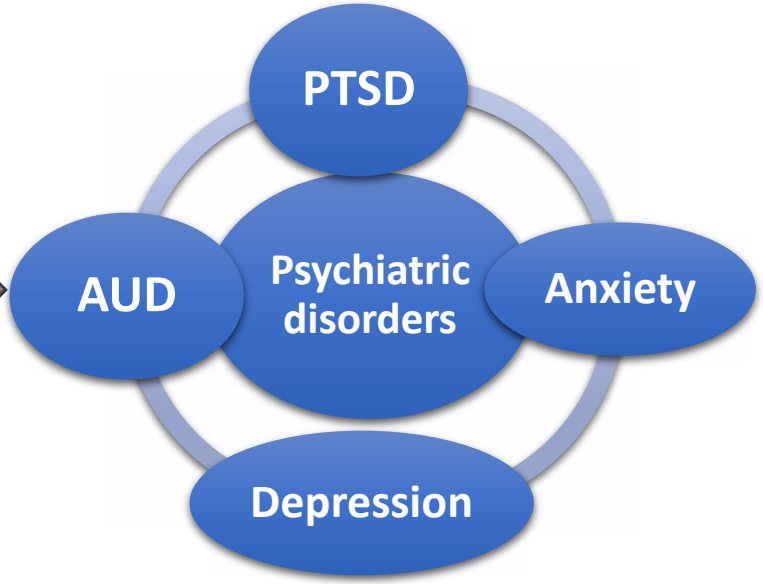
Witness stress

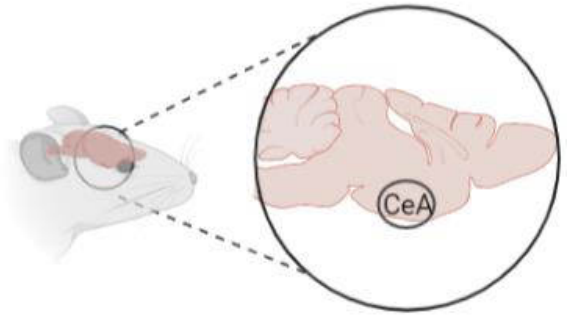
Dysregulation of the CRF system and HPA axis

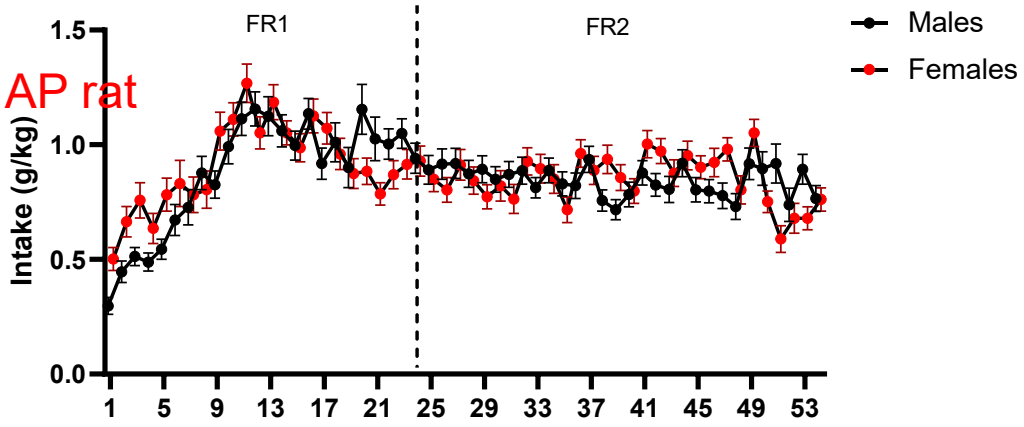
Maternal Separation

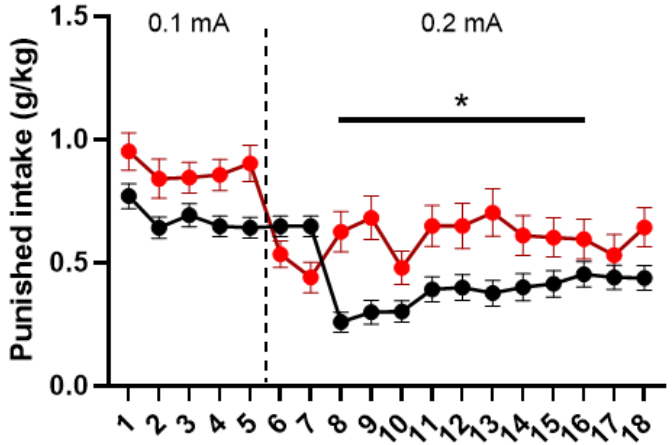
Maladaptive behaviors

Risk factor







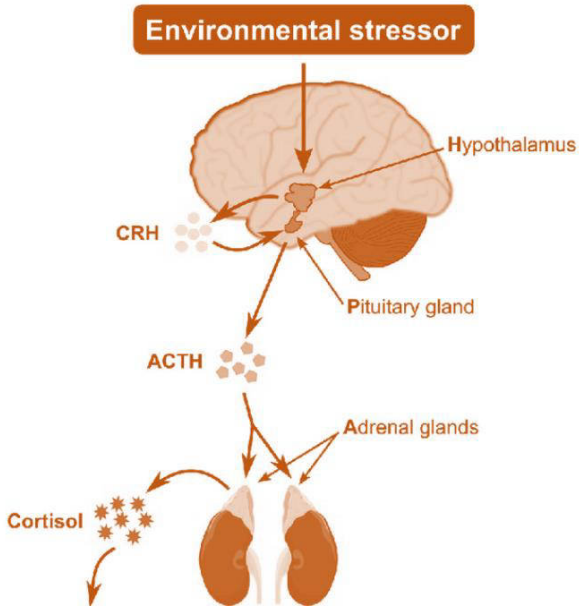


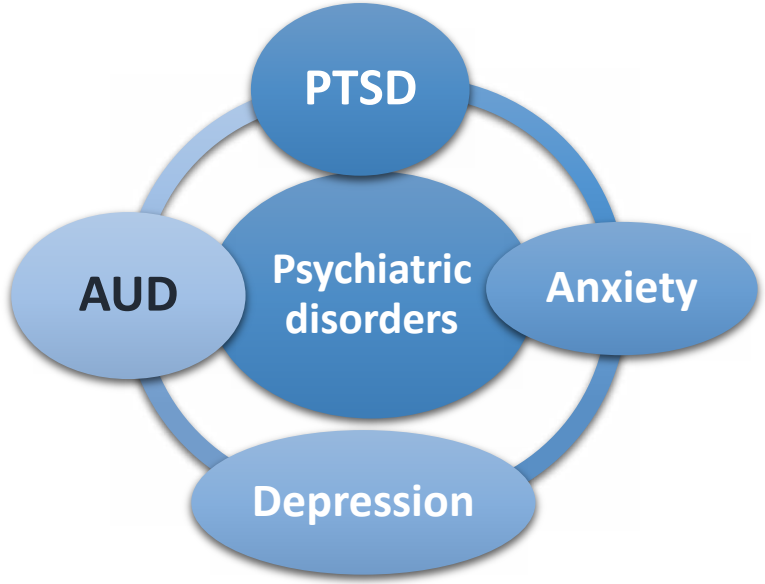
Genome-wide methylation in alcohol use disorder subjects: implications for an epigenetic regulation of the cortico-limbic glucocorticoid receptors (NR3C1)

Early life stress is an important environmental risk factor for the development of AUD

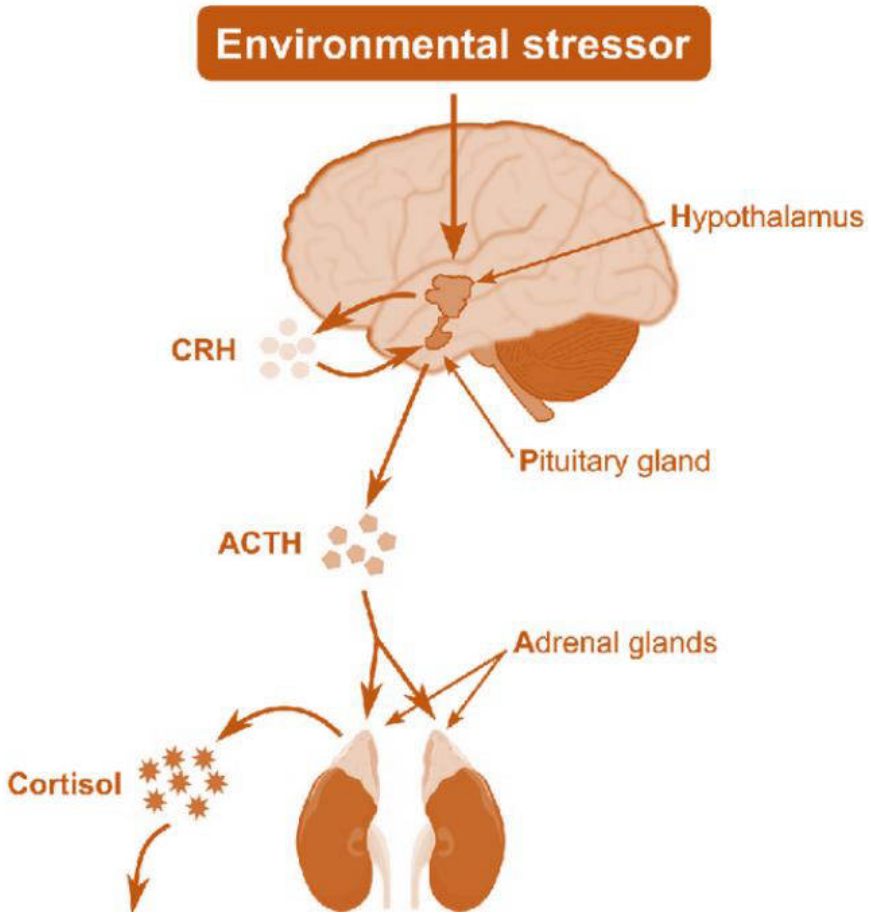
Maternal Separation

Maladaptive behaviors

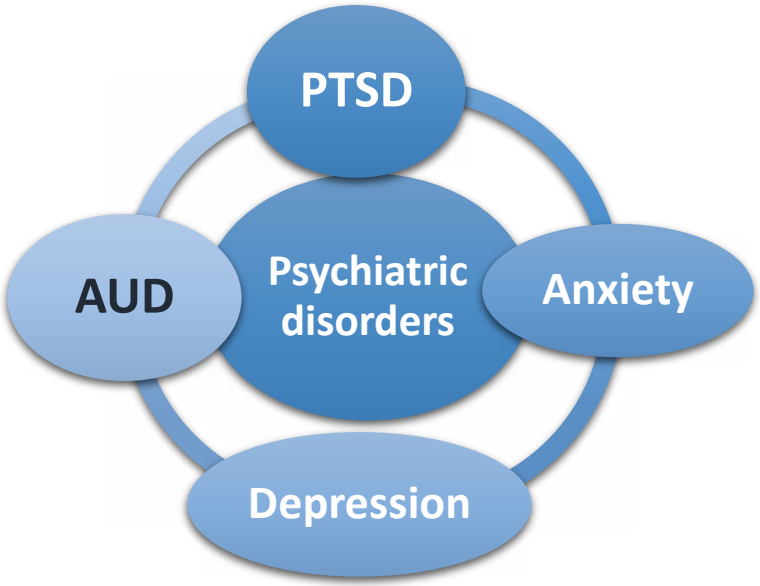




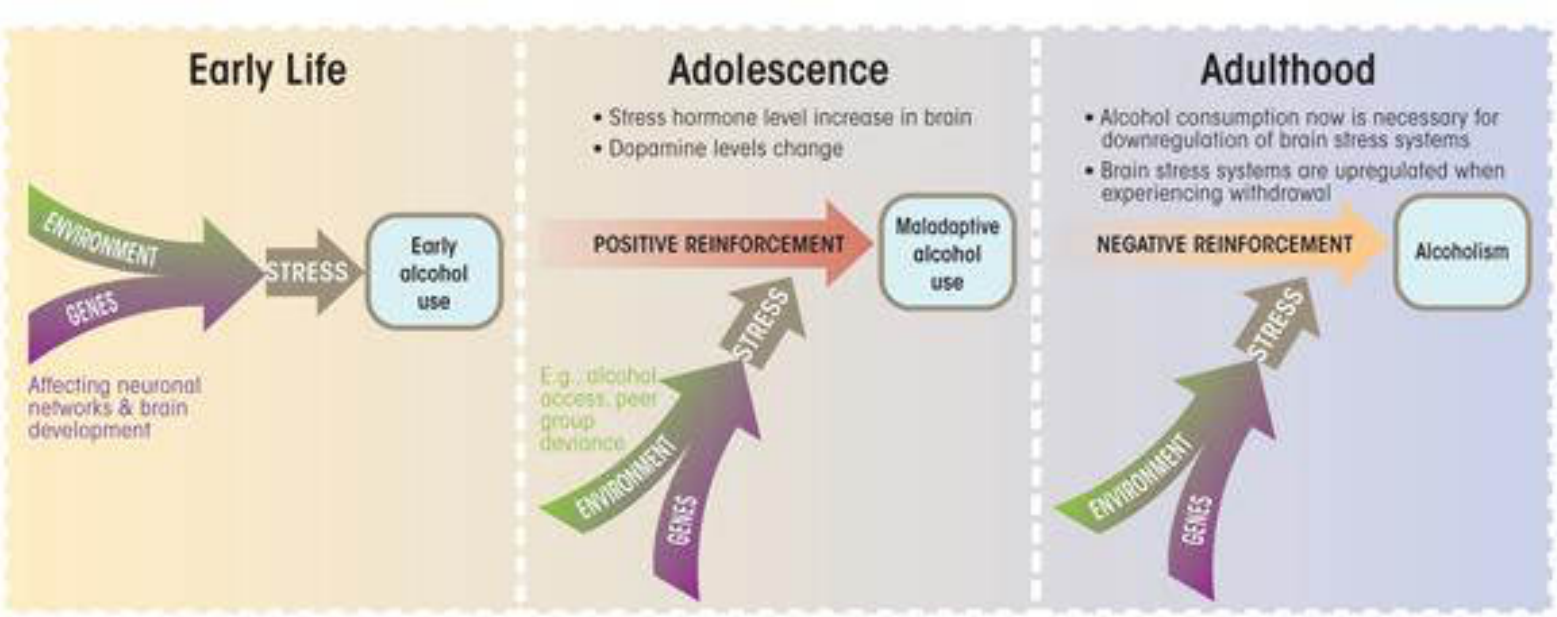
Stress is an important environmental risk factor for the development of AUD



Maladaptive behaviors



Early life stress is an important environmental risk factor for the development of AUD



Evaluate the effects of ESI on Wistar and genetically selected msP rat lines

Stress, dysregulation of the HPA axis and alteration in glucocorticoid receptor (GR) function have been associated to the transition to alcohol dependence and play an essential role in modulating negative affective states *(Stephens and Wand, 2012; Edwards et al., 2015)*