

# Prenatal alcohol exposure affects the early postnatal stress and immune hypo-responsive periods.

Andrea Allan, Erin Milligan, Shahani Noor, Estrella Villicana, Jane Smoake and Kevin Caldwell

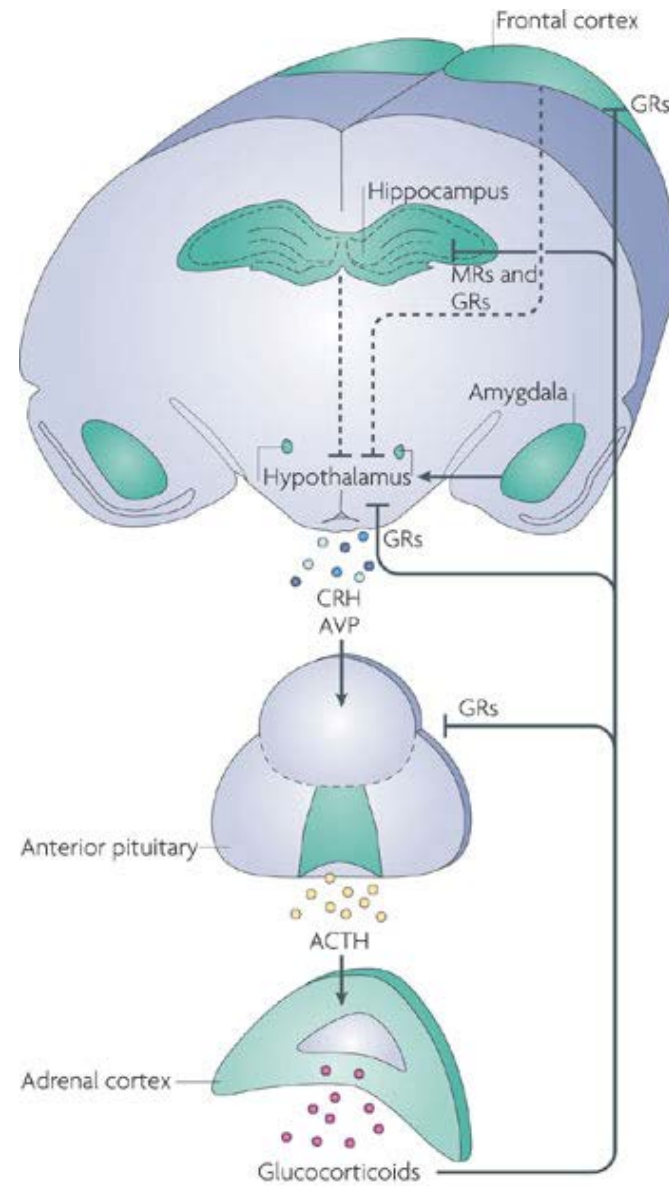
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## DEFINITION OF Stress Hypo- Responsive Period (SHRP)

- Period in early development that is marked by HPA suppression.
- Reduced stress response seen during ~ first 2 weeks postnatal life (PND1-10 in mouse).
- Stressful stimuli evoke a subnormal HPA response.
- GC plasma levels are lower than predicted.
- Early prenatal and postnatal exposure to high levels of GCs have been implicated in disruption of the SHRP and increasing GC resistance

GC Resistance is a process of the CNS.

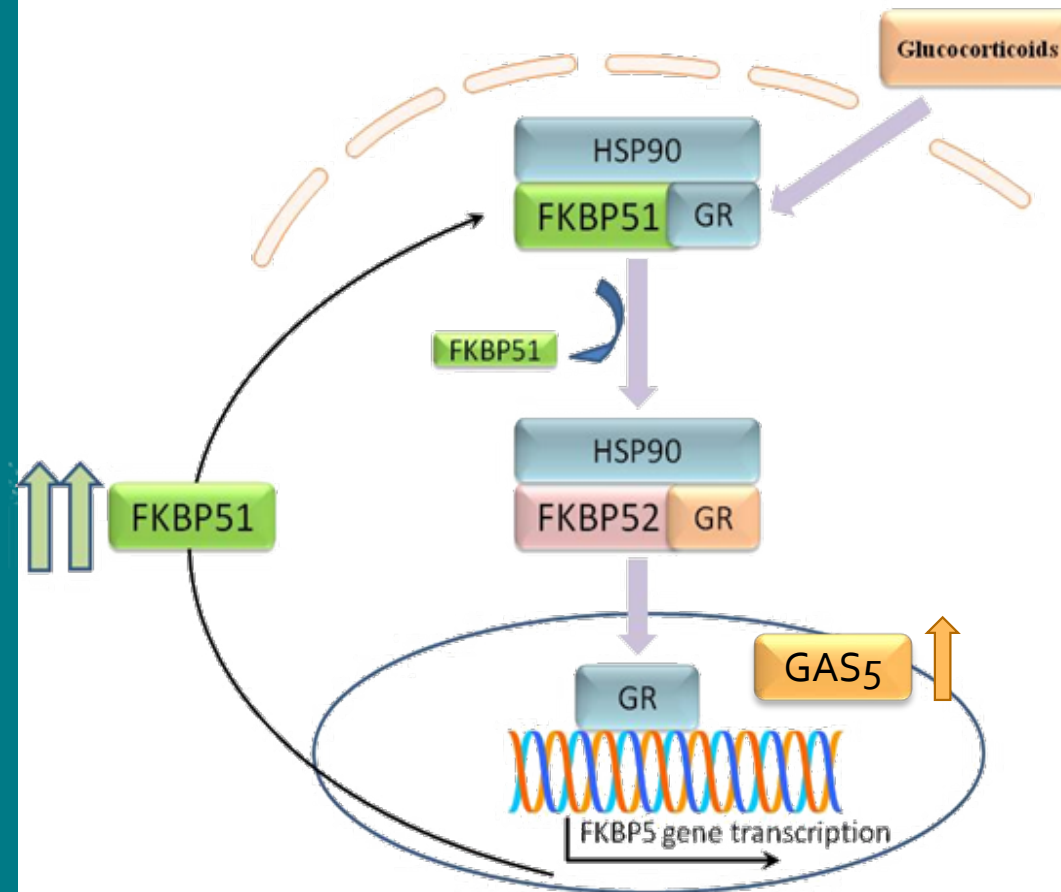
Many of the same consequences seen in FASD.



Prenatal alcohol exposure has been associated with many of the same pathological conditions described in GC resistance:

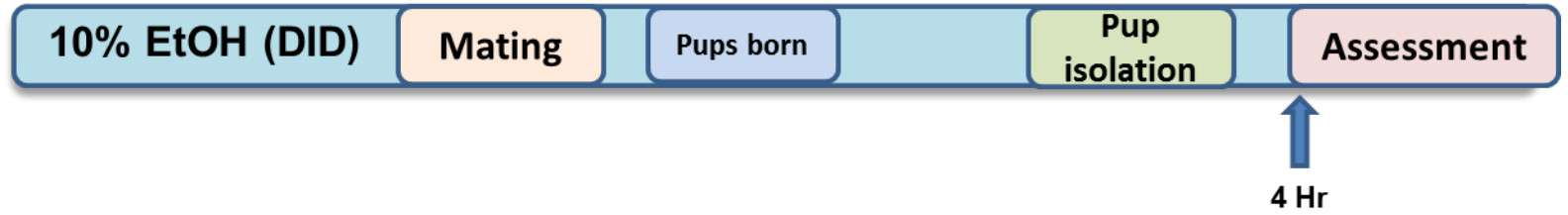
- asthma (Magnus et al., 2014; Wada et al., 2016)
- metabolic syndrome (Dobson et al., 2014; Fuglestad et al., 2014; He et al., 2015; Vaiseram 2015)
- insulin resistance (Chen and Nyomba 2004; Harper et al., 2014; Nammi et al., 2007; Shen et al., 2014; Soscia et al.2006;Yao et al., 2008)
- glucose intolerance (Gardebjer et al., 2015; Yao et al., 2013; de la Monte 2010; Yao et al., 2007)
- depression (Caldwell et al., 2008; Cryan, Markou, & Lucki, 2002; O'Connor and Paley, 2006; Streissguth et al., 1996; Winsper et al., 2015)
- inflammatory disorders (Carson et al., 2012; Komada et al., 2017; Wada et al., 2016)
- immune disorders (Pascual et al., 2017; Raineiki et al., 2017; Zhang et al., 2012)
- increases in inflammatory cytokines (Bodnar et al., 2016;Lee and Rivier, 1996; Noor et al., 2017; Raineiki et al.; 2017; Toso et al., 2006; Teraski et al., 2016; Vink et al., 2005).

# Mechanisms of GC Resistance



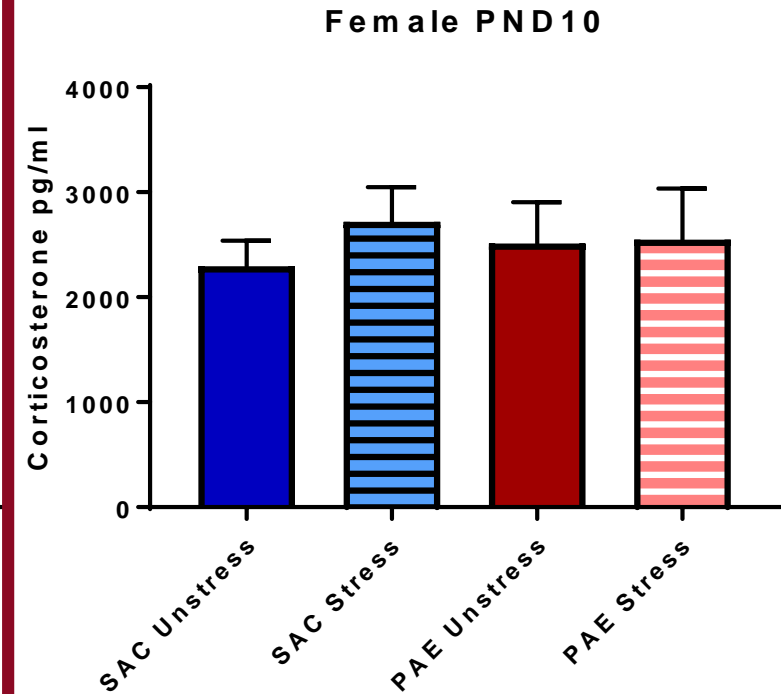
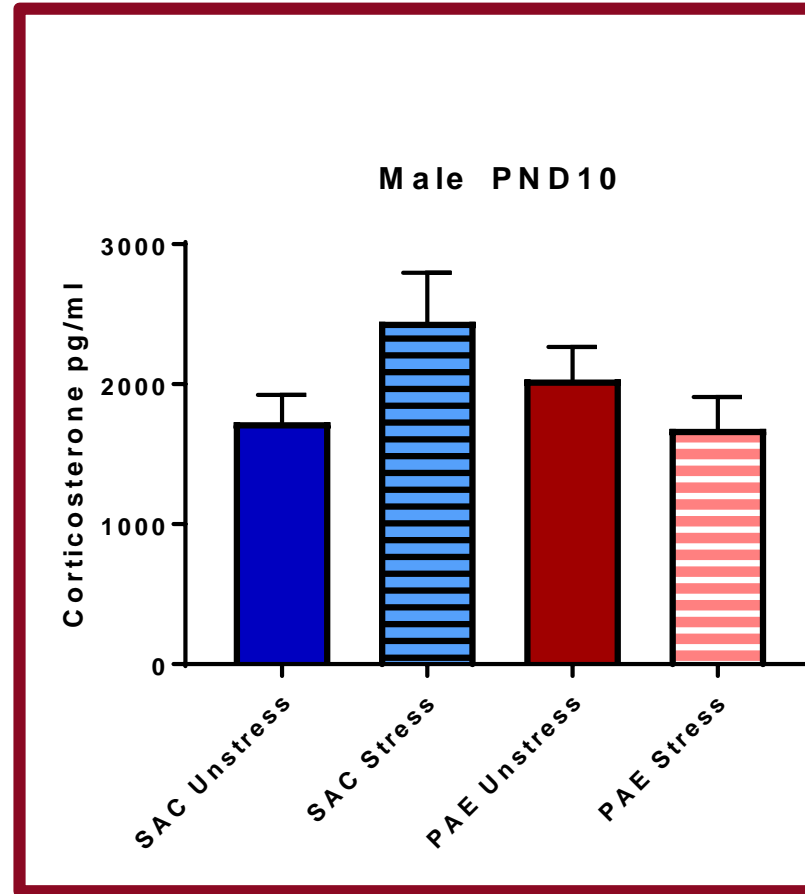
- ↑ Growth arrest-specific transcript -5 (Gas5) lncRNA
- ↑ miR-124
- ↓ GR $\alpha$  : GR $\beta$  balance
- ↑ GR phosphorylation state
- ↑ FK506-binding protein 51 (FKBP51) levels
- ↓ Heat shock protein 90 (Hsp90)

# Our SHRP protocol

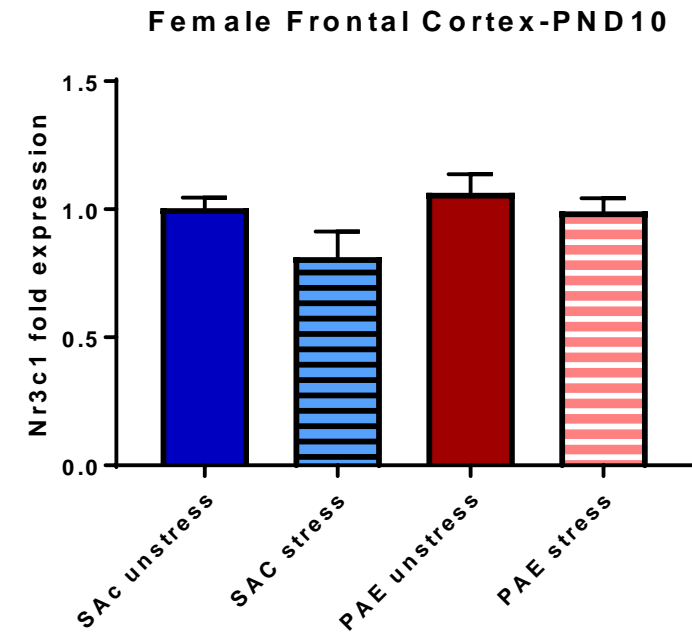
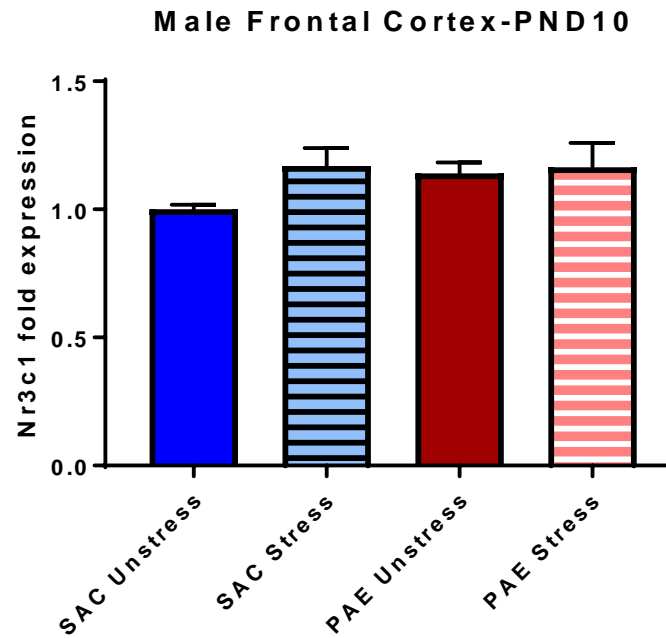


- ✓ Drinking is during 4 hours in the active period under reverse light dark cycle.
- ✓ Blood alcohol is ~80-90 mg% on average
- ✓ Pups are removed from dam (stressed condition) and located in a different vivarium
- ✓ Control (unstressed groups) are left with dam and killed at the same time as the stressed group
- ✓ Brain and plasma prepared and snap frozen until time of assay.
- ✓ mRNA determined using qPCR and Meso Scale Discovery.

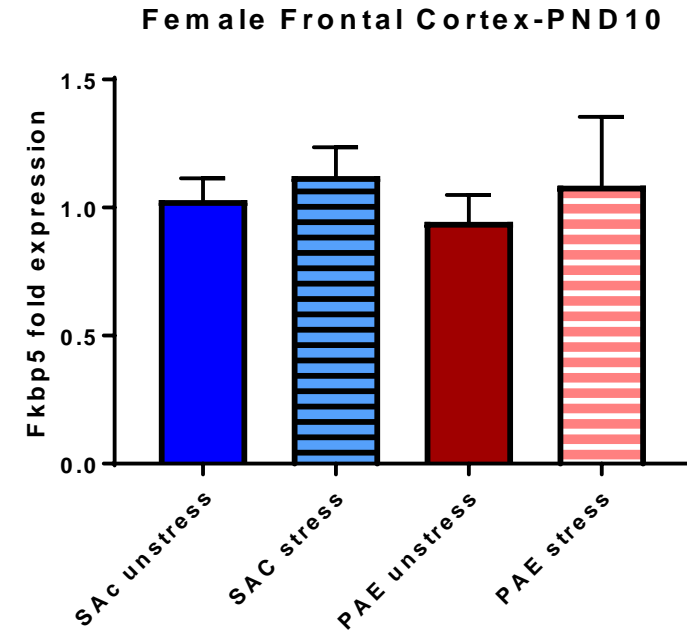
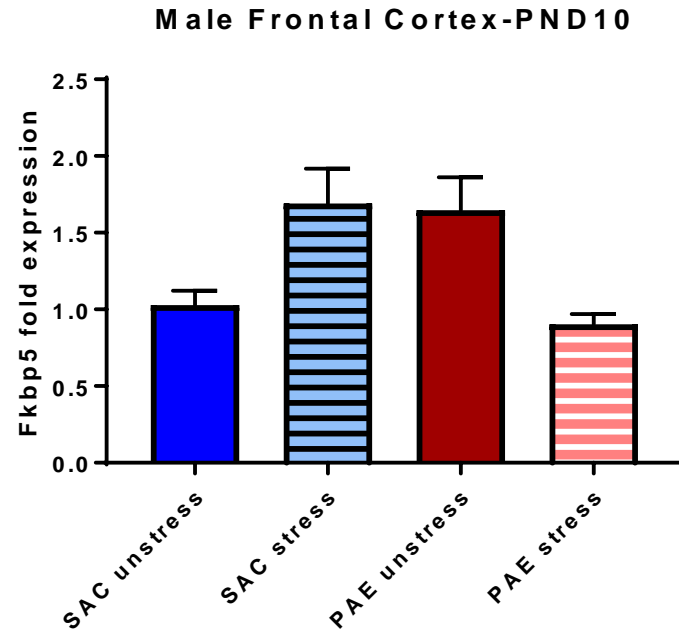
# Plasma corticosterone levels increased by maternal separation in male SAC



Nr3c1 levels are increased in SAC stressed males but not PAE males. Females do not have much of a response

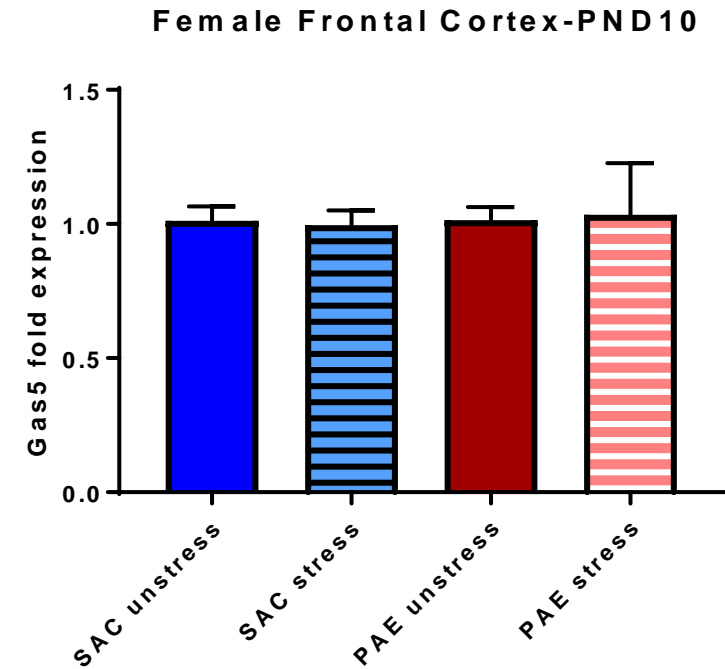
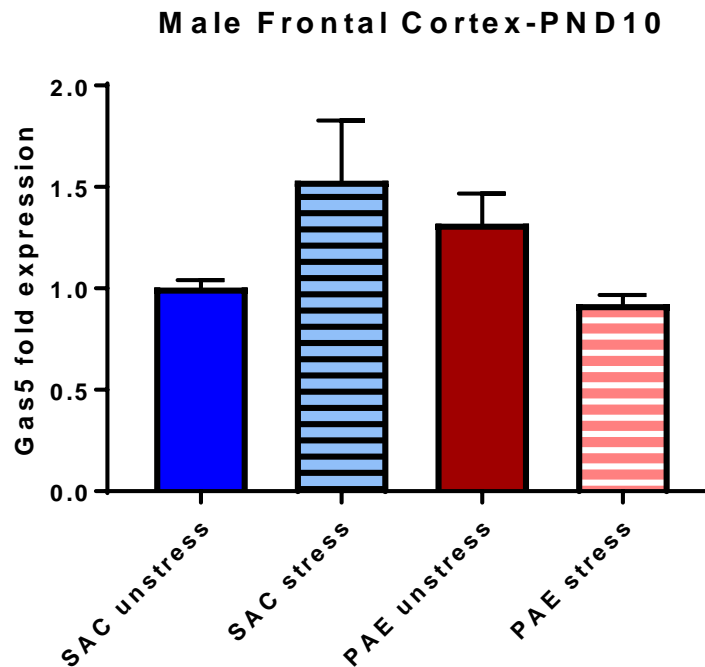



Fkbp5 is increased in SAC males but decreased in PAE following maternal separation





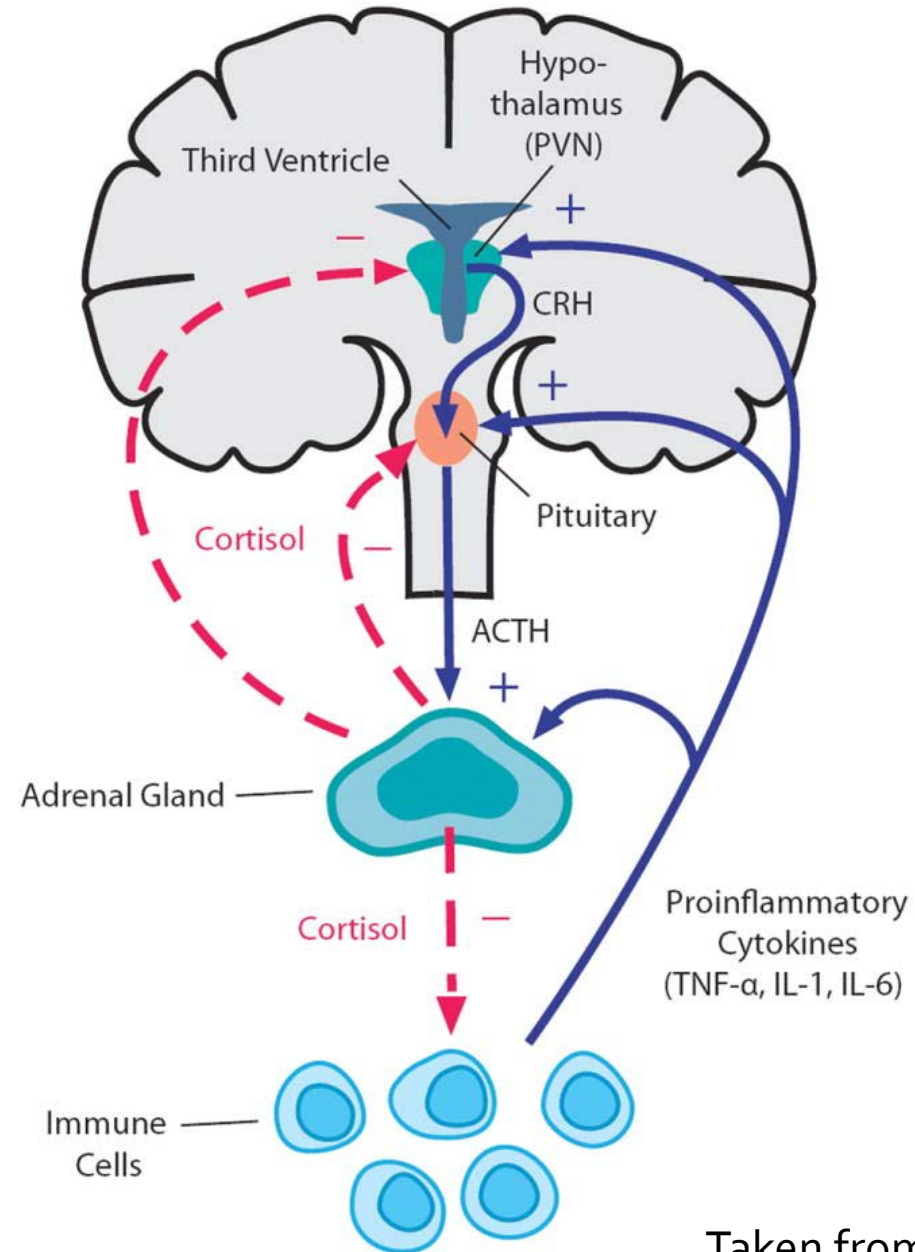
Gas 5 levels are increased in SAC but decreased in PAE following maternal separation





What is the link between  
the immune system and  
the stress pathway?

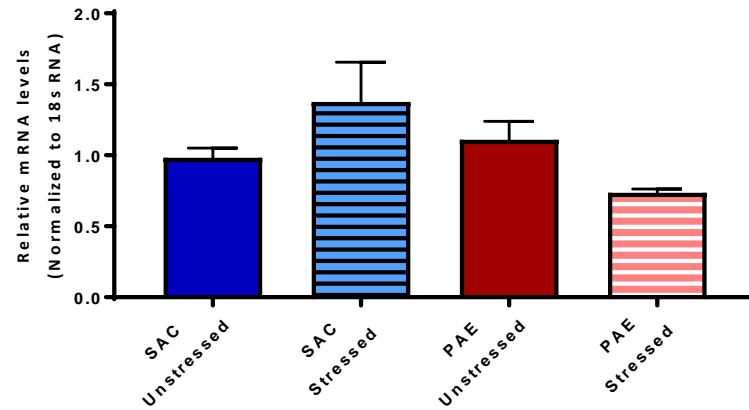
There is a bidirectional communication between the immune system and the HPA axis



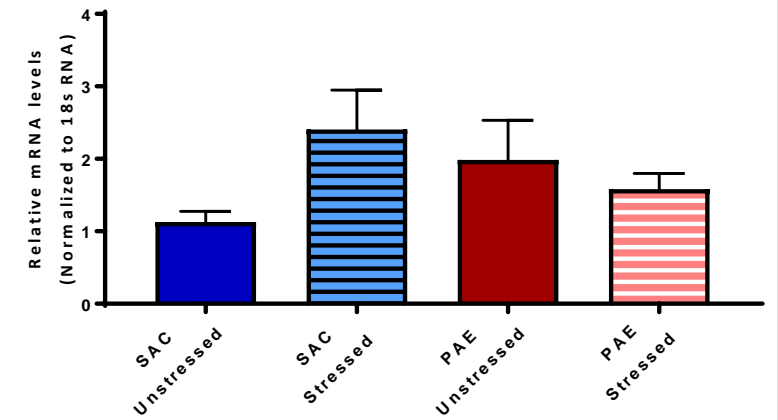
Taken from Silverman et al., 2003)

Inflammatory cytokines are increased in SAC following maternal separation but are decreased in PAE males

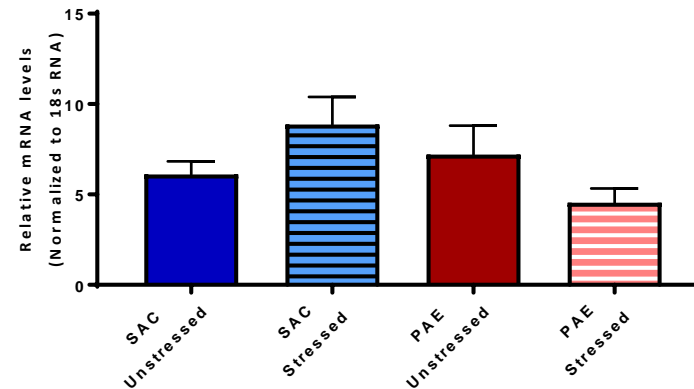
TNF- $\alpha$  Hypothalamus



IL-10-Hypothalamus

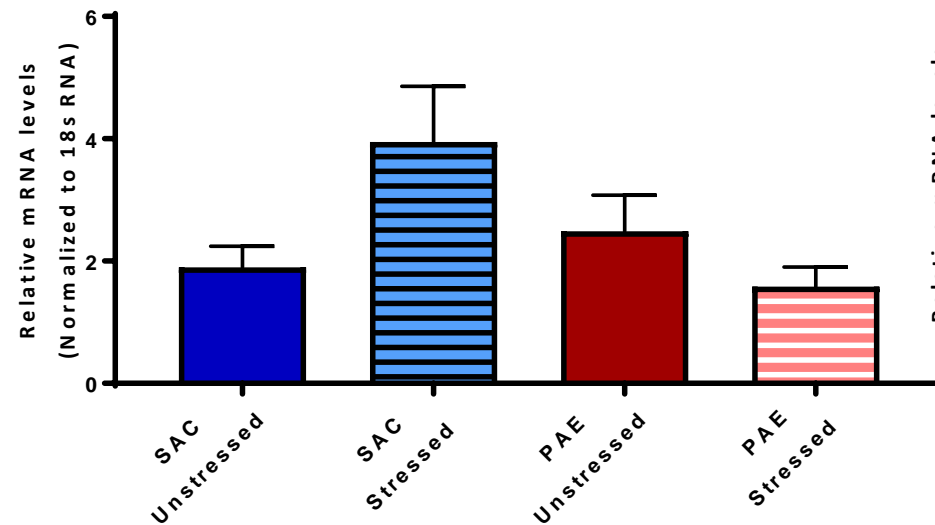


IL-1 $\beta$  Hypothalamus

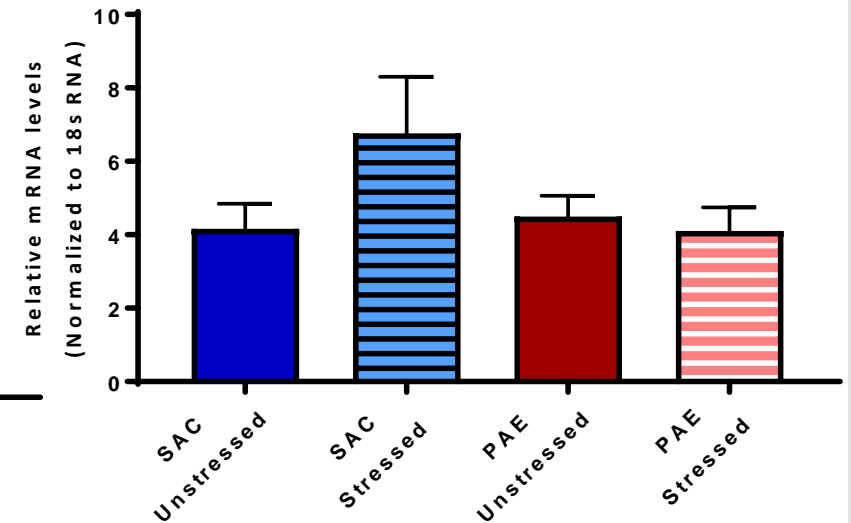


Inflammatory markers are elevated in SAC stressed but not in PAE stressed males.

GFAP-Hypothalamus



CCL2-Hypothalamus



# Conclusions

- Prenatal exposure to alcohol alters the development of the glucocorticoid system
- Stress hyporesponsive period as measured by plasma corticosterone, appears to be nearly completed at PND 10 in the SAC under this protocol
- There is an increase in mRNA expression of nr3c1, fkbp5 and Gas5 following maternal separation in the SAC males, but decreases in fkbp5 and Gas 5 are seen in PAE males.
- SHRP is present in PAE males but female PAE mice do not display an SHRP response
- Females (both PAE and SAC) appear to be less affected by the separation at the time point tested.
- PAE males may display a longer SHRP than SAC males. Little to no SHRP is seen in the females tested. Additional PND timepoints need to be assessed.
- Inflammatory cytokines and markers are increased in SAC following maternal separation but are mostly decreased or unchanged in PAE males.

## So what next ?

- If Prenatal alcohol alters the internal programming of glucocorticoid regulation leading to a myriad of negative health outcomes, what can we do about it?
- Can we use targeted miR modifications to increase GC sensitivity?
- Are there behavioral modifications that can be applied?
- In patients with FASD should we be more aggressively managing the potential health risks of glucocorticoid resistance?
- If we fix the inflammatory/immune dysregulation then will we have a fix the glucocorticoid resistance and vice versa