Sex differences in biochemical but not behavioral responses to delay fear conditioning: effects in control and prenatal alcohol-exposed mice

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OUTLINE

Introduction

Behavioral studies

Biochemical studies

Conclusions

INTRODUCTION

- Mouse model of prenatal alcohol exposure
- Fear conditioning and behavioral measures

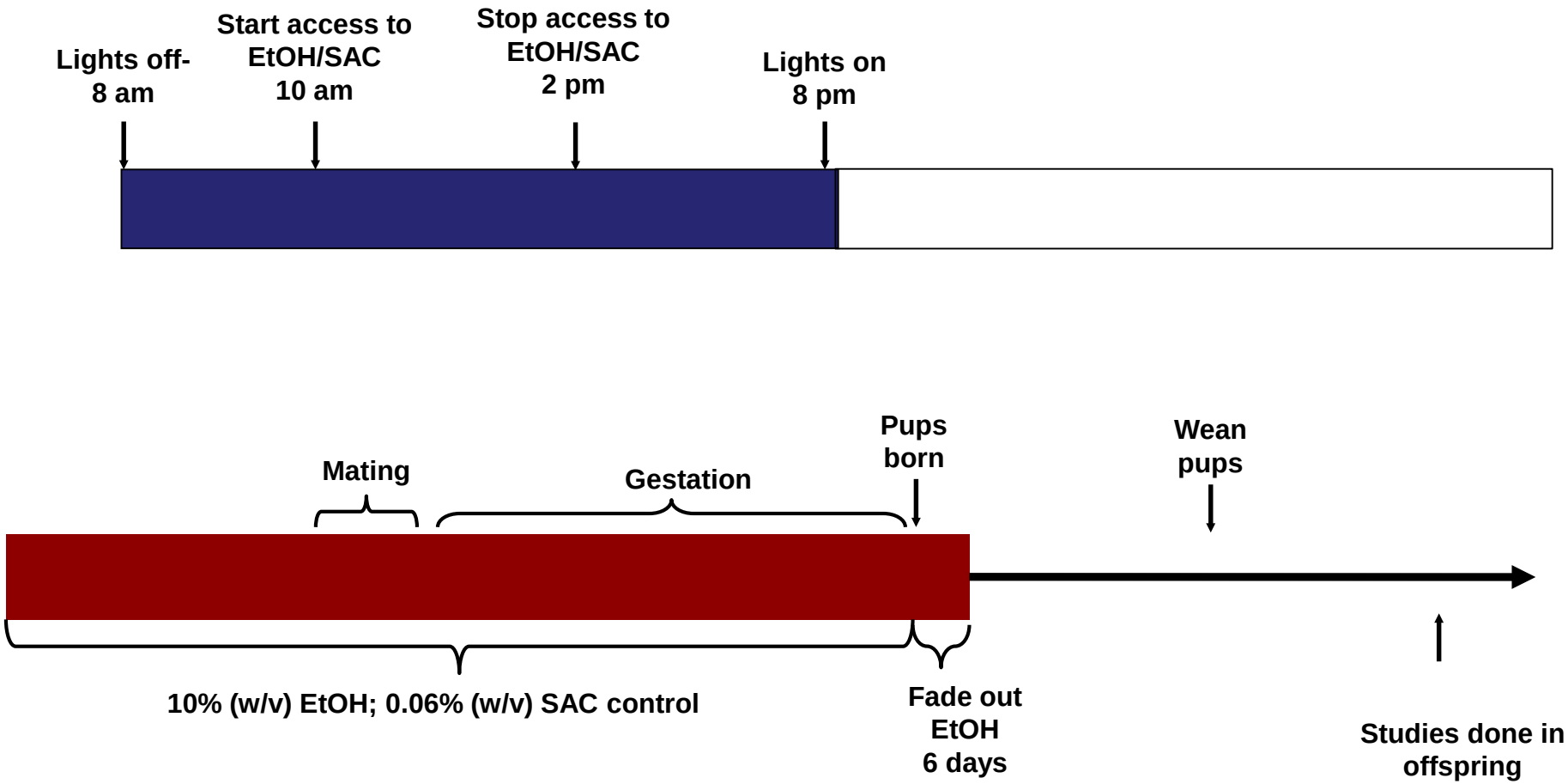
Considerations for prenatal ethanol exposure rodent models

- Influence of genetic background
- Blood ethanol concentrations that are desired and how this impacts the route of administration
- Length, pattern/frequency and developmental timing of the exposure

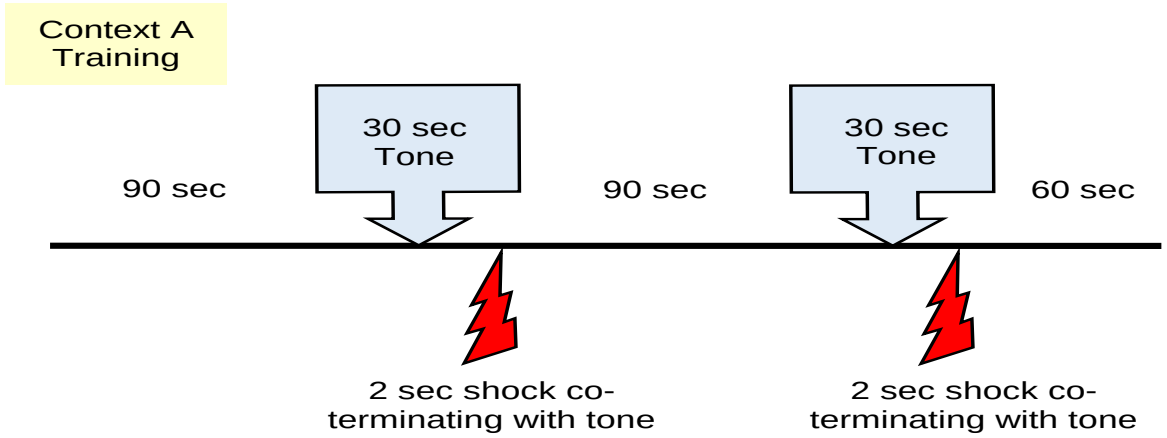
Drinking-in-the dark model (2012 – present)

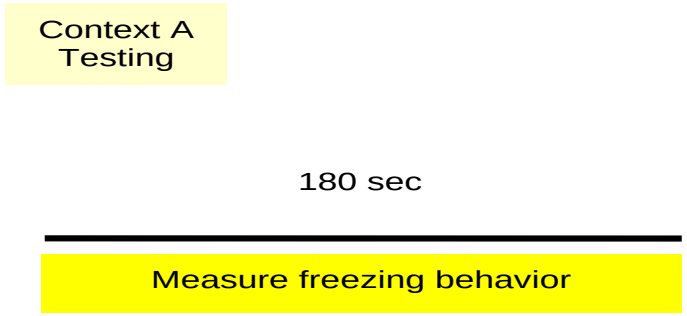
- access to ethanol / saccharin during a limited (4h) drinking session starting 2h into dark cycle
- 10% (w/v/) ethanol in 0.06% (w/v) saccharin; control 0.06% (w/v) saccharin
- C57BL/6J mice (Jackson Laboratories)
- BEC: 80 – 90 mg/dL after four hours of drinking

Prenatal exposure paradigm



Delay fear conditioning



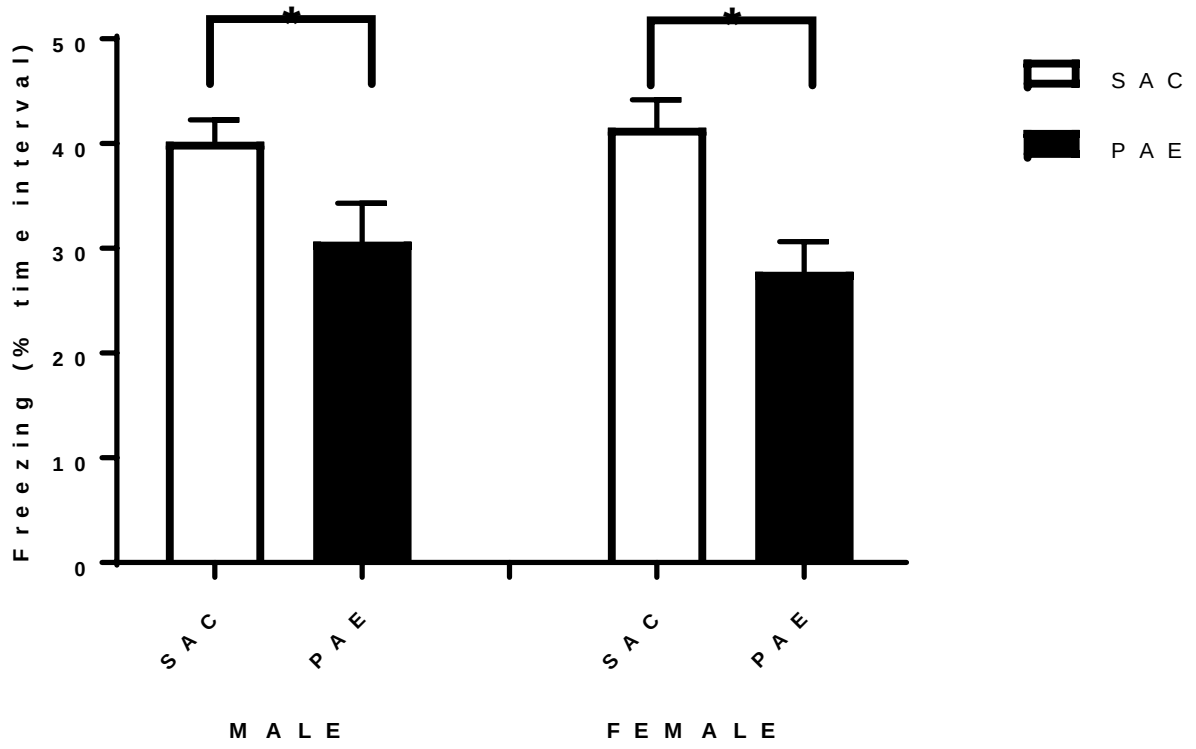


The utility of single-trial fear conditioning in the study of the effects of PAE

- High temporal resolution
- Neuronal circuitry relatively well defined
- Deficits in males have been shown to be reproducible across PAE models

Do PAE females display a deficit in contextual fear conditioning?

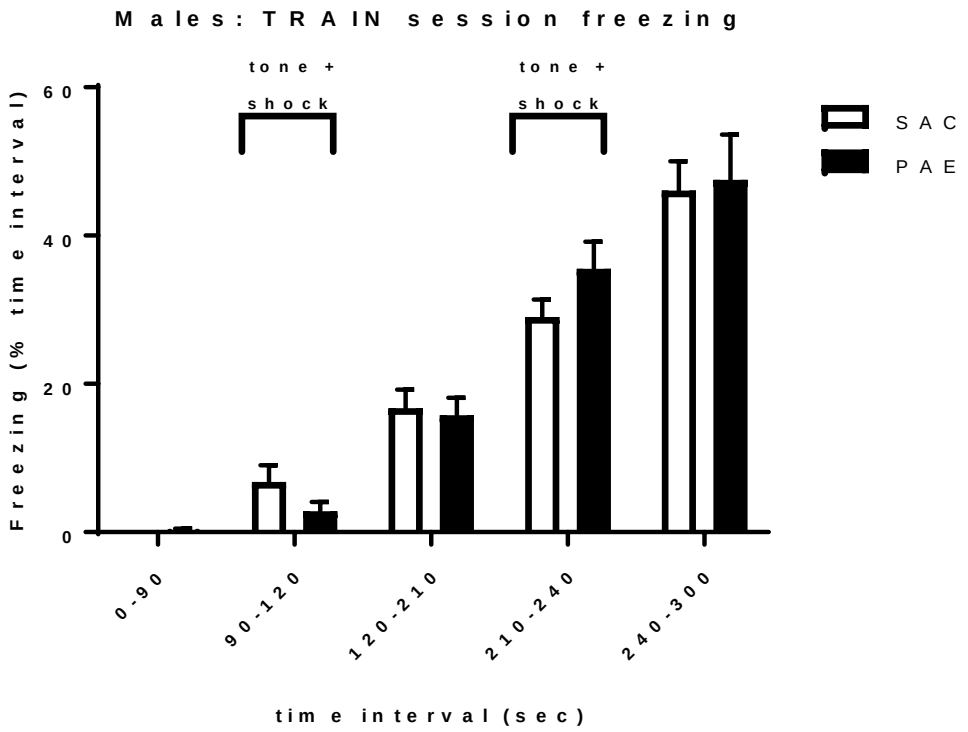
Male and female PAE mice display a deficit in contextual fear memory recall

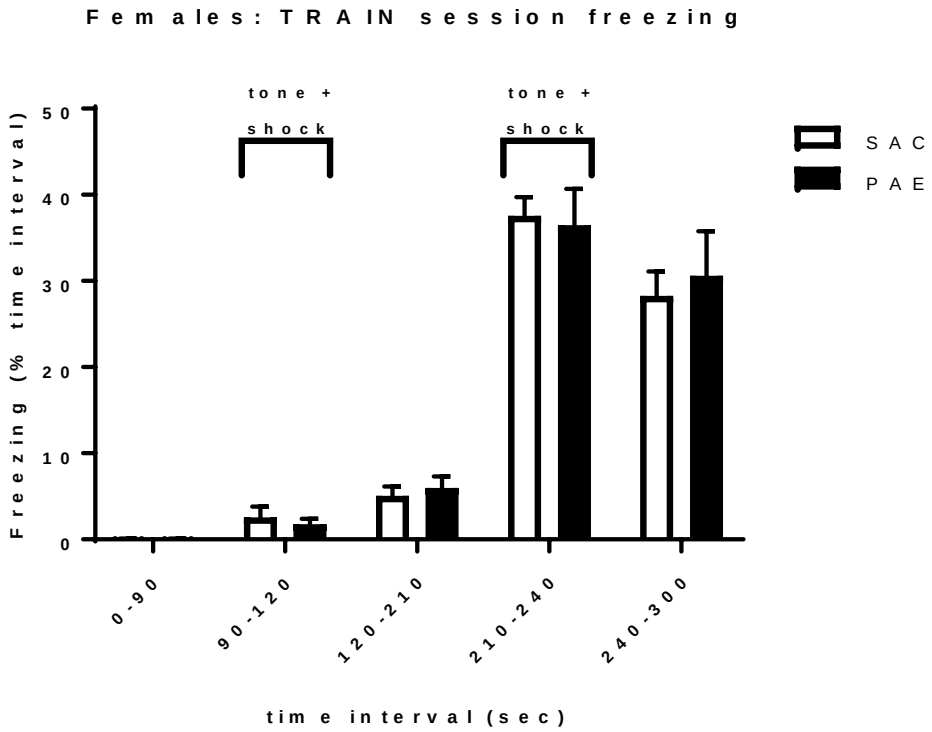


Is the observed contextual fear deficit due to:

1. **an inability of the PAE mice to produce the fear (freezing) response?**
2. **A deficit in the acquisition of information during conditioning?**

SAC and PAE mice display similar fear responses during conditioning





BEHAVIORAL STUDY CONCLUSIONS

- SAC and PAE animals do not differ in their ability to respond to and recognize the training experience as being fearful.
- However, they display a deficit in their ability to form (consolidate) a memory of the experience and/or their ability to recall the experience, when presented with reminders, at a later time.

Are there biochemical alterations that correlate with the observed behavioral deficits?

Glucocorticoids and the HPA axis

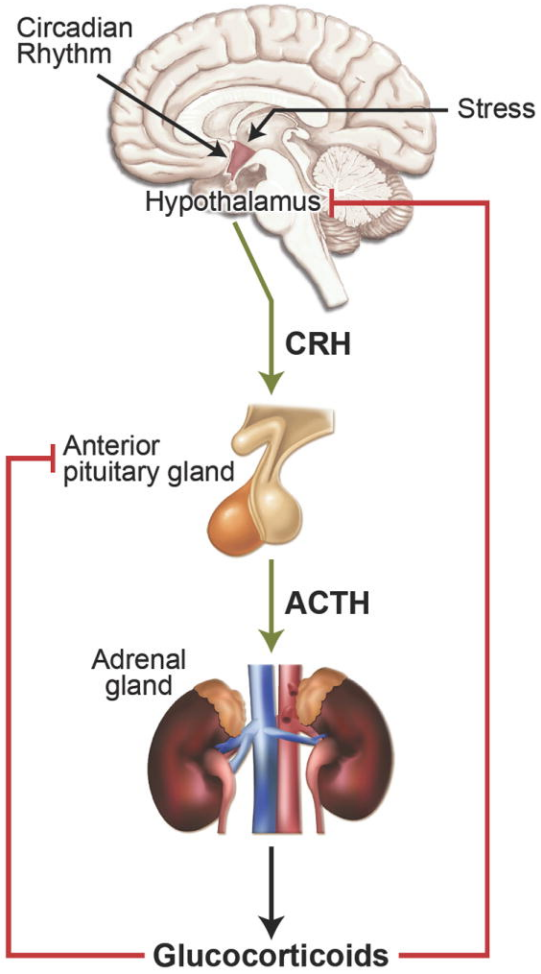
Glucocorticoids (GCs) are released by the adrenal cortex in a circadian manner and in response to stress.

Secretion of GCs is controlled by the hypothalamic-pituitary-adrenal (HPA) axis.

GCs act on nearly every tissue and organ in the body.

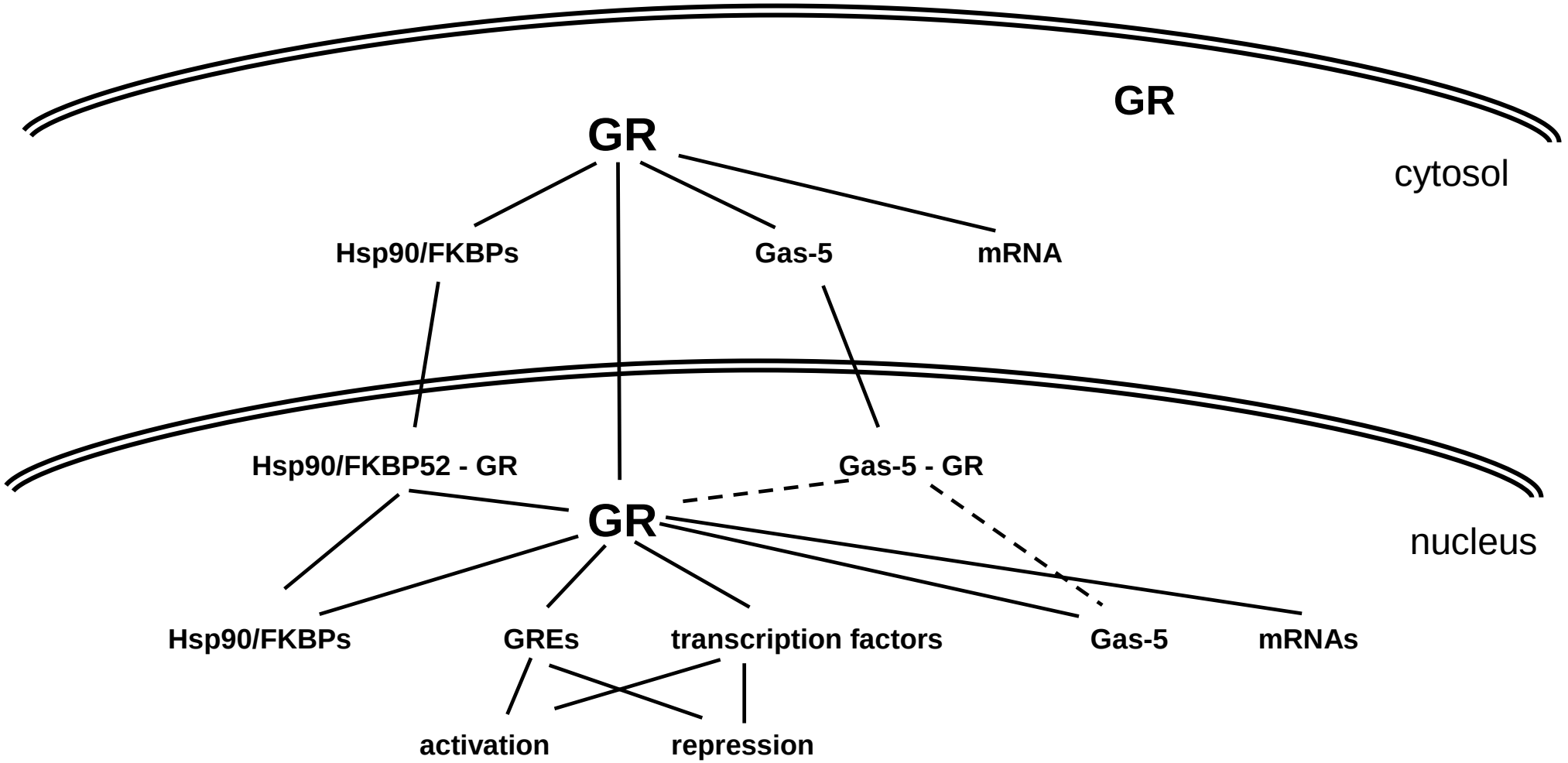
GCs regulate a plethora of physiological processes including intermediary metabolism, immune function, CV function and cognition.

GR system is programmed in utero, during the alcohol exposure period.



Oakley & Cidlowski 2013J Allergy Clin Immunol.

How does the GR exert its effects?



What is known about the role of the GR in fear-conditioned learning, memory and memory recall?

GR IN LEARNING / MEMORY CONSOLIDATION – **all studies in males**

Peripheral (sc) injection of RU 38486 (GR antagonist) either 1 hour before or immediately after tone-shock conditioning reduced contextual fear in rats. (Pugh et al. 1997)

Inverted U-shape DRC for the effect of peripherally (sc or ip?) administered corticosterone given administration immediately after training on contextual fear responding measured 24 h later (Abrari et al. 2009)

Effects of corticosterone infused into the dorsal hippocampus immediately after conditioning depend on stressor intensity: increased context conditioning at lower (0.3 mA) shock intensity but impaired context conditioning at higher (0.8 mA) shock intensities (Kaouane et al 2012 Science).

ICV injection (Cordero and Sandi 1998; Donley et al. 2005) or injection into ventral hippocampus (Donley et al. 2005) of RU 38486 45-60 min prior to training reduced context fear expression measured at 24 hr after training; effect seem at intermediate shock intensity but not at stronger shock intensity; no effect of ICV infusion of MR antagonist (Cordero and Sandi 1998).

GR IN LEARNING, MEMORY CONSOLIDATION AND/OR RETRIEVAL - **males**

GR knockdown in adult-born neurons in mouse DG reduced context freezing measured 24-hr after training; could be an effect on learning, memory consolidation or retrieval.

GR IS NOT IMPORTANT FOR CONTEXTUAL FEAR RETRIEVAL/EXPRESSION -**males**

treatment (s.c.) treatment with RU486 one-hour prior to the 24-hr context test had not effect on contextual fear. (Zhou et al. 2011).

CONCLUDE: In males, GR is important for contextual fear learning/memory consolidation but does not appear to play a role in contextual fear memory retrieval.

Experimental Design

PND 180 males and females

female estrous cycle: Whitten effect; stage: diestrus for training

delay fear conditioning

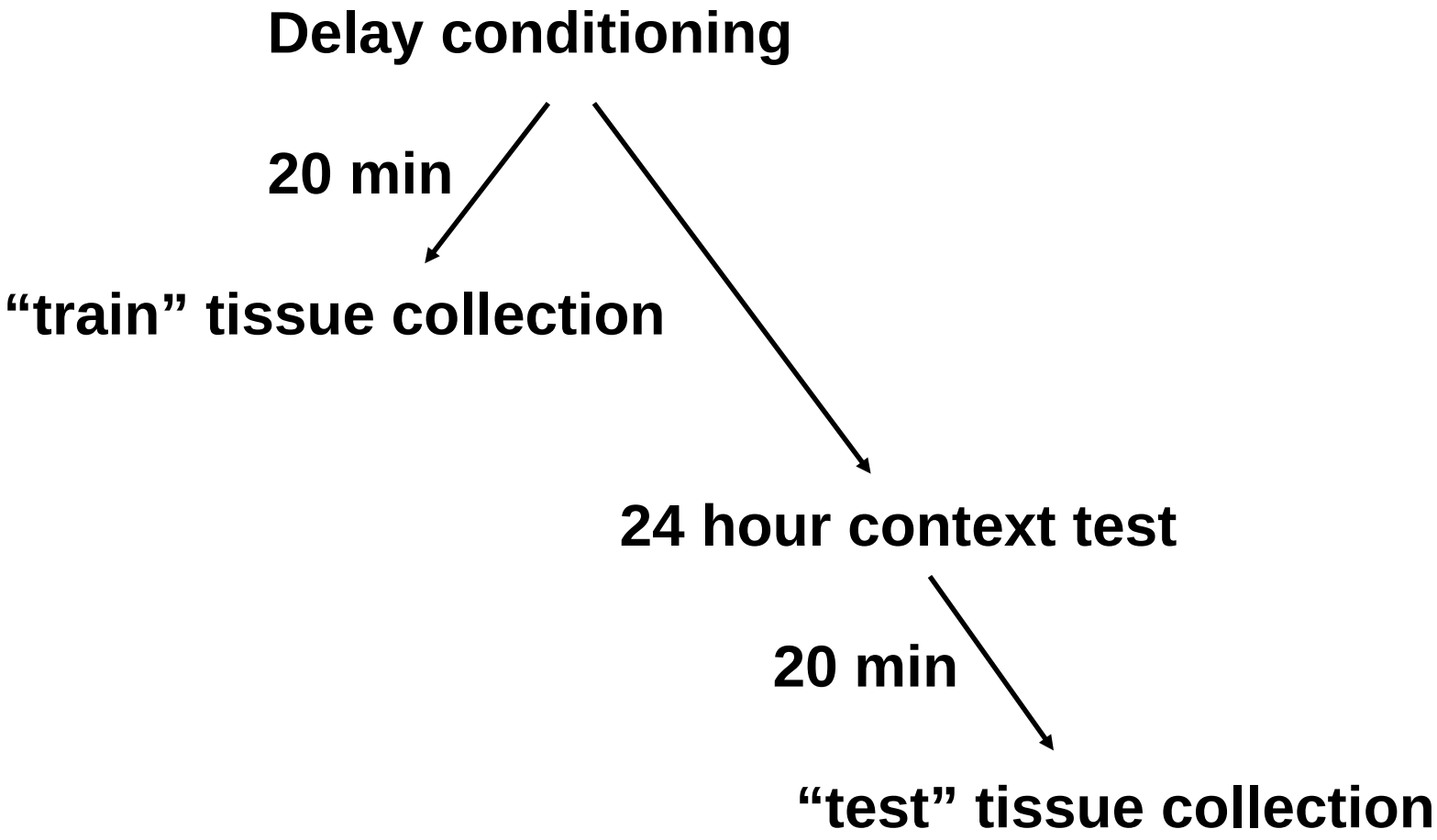
3 groups: naïve, train (tissue collected 20 min after training) and test (tissue collected 20 min. after testing)

collect blood and isolate HPF from brain

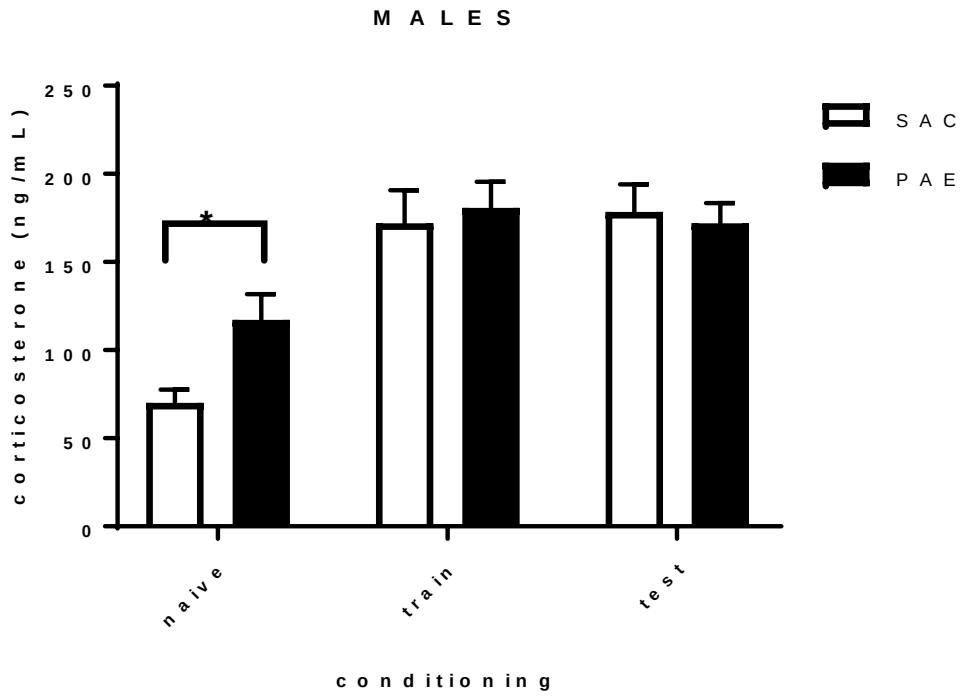
prepare subcellular fx. (nuclear, cyto, PM)

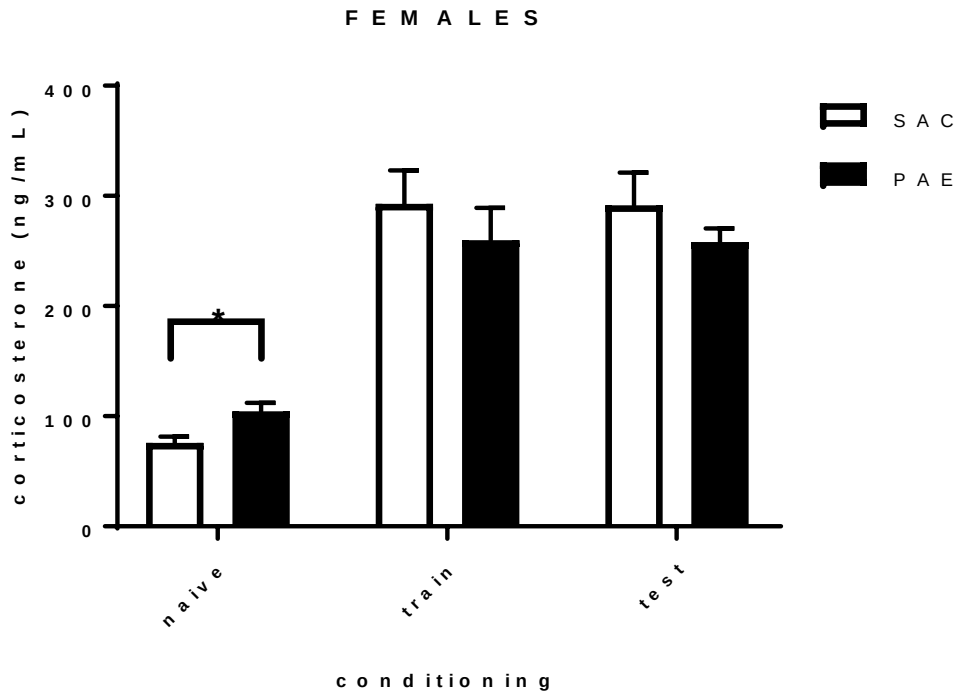
protein levels: immunoblotting

Delay conditioning, testing and tissue collection

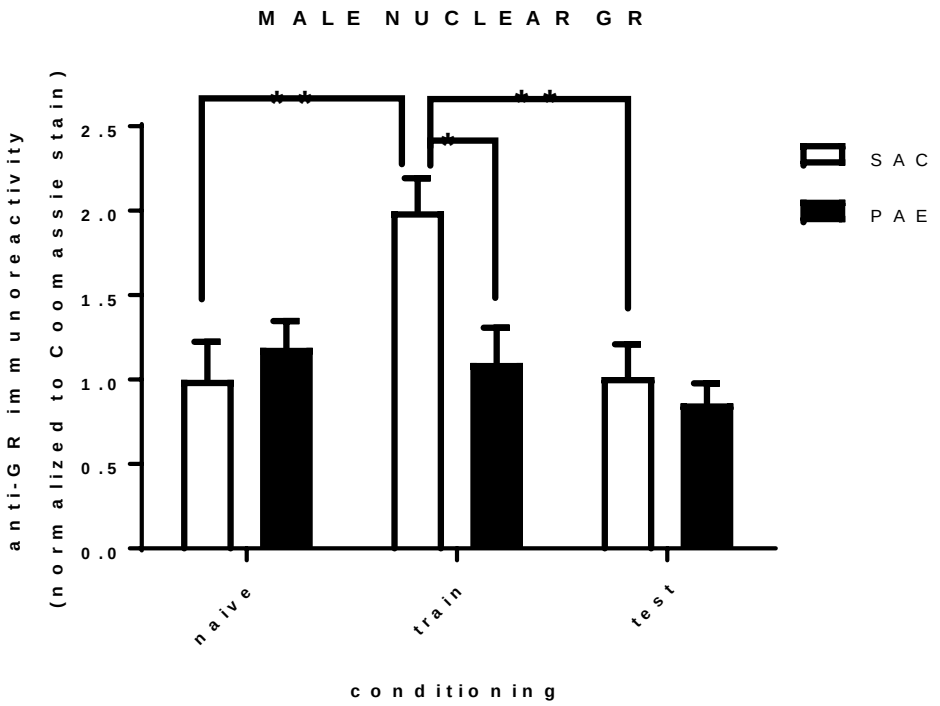


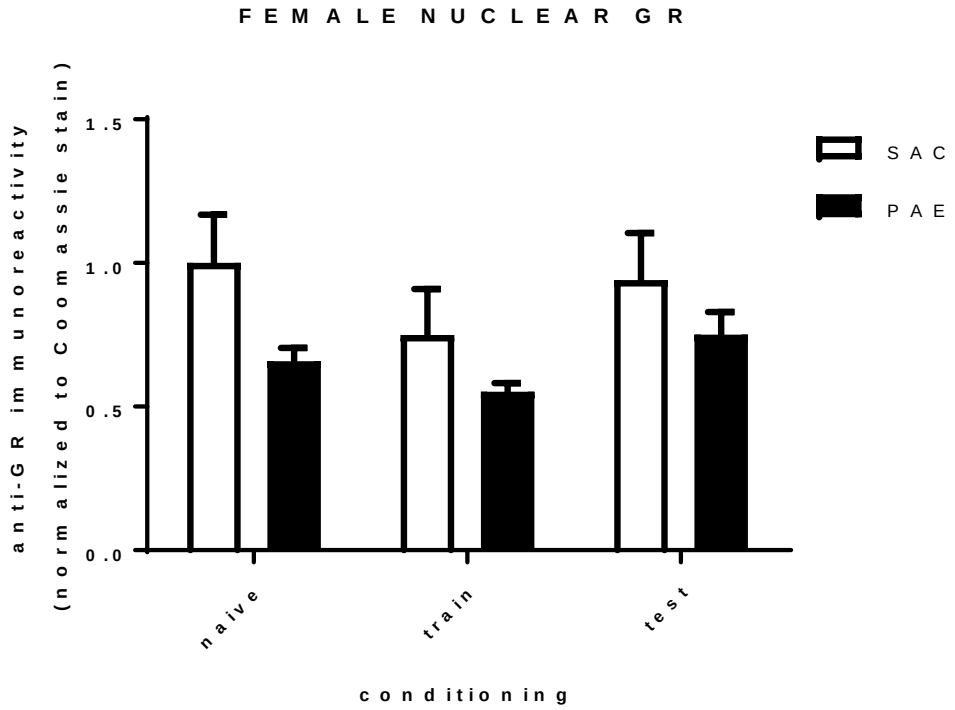
Effects of PAE and fear conditioning on plasma corticosterone levels





Nuclear GR is increased following fear conditioning (training) in SAC, but not PAE, males





Mechanisms that may underlie the persistent reduction in nuclear GR levels in PAE females

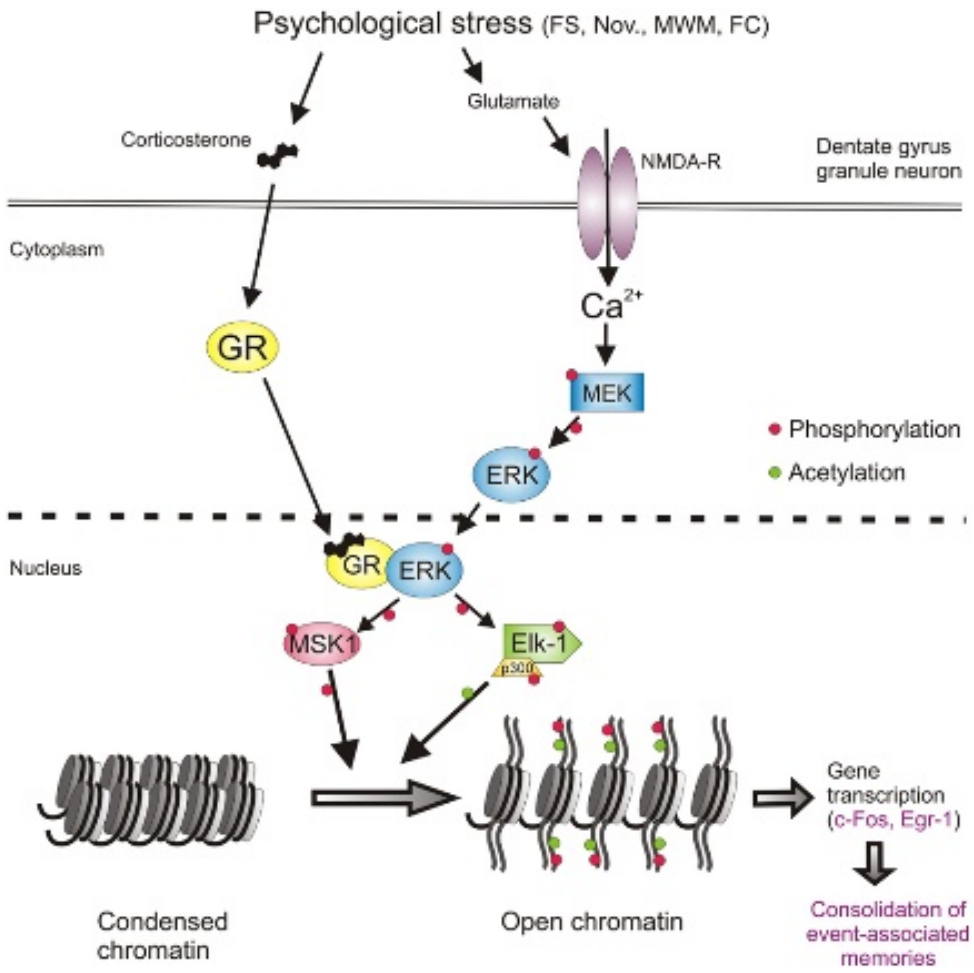
Dysregulation of trafficking

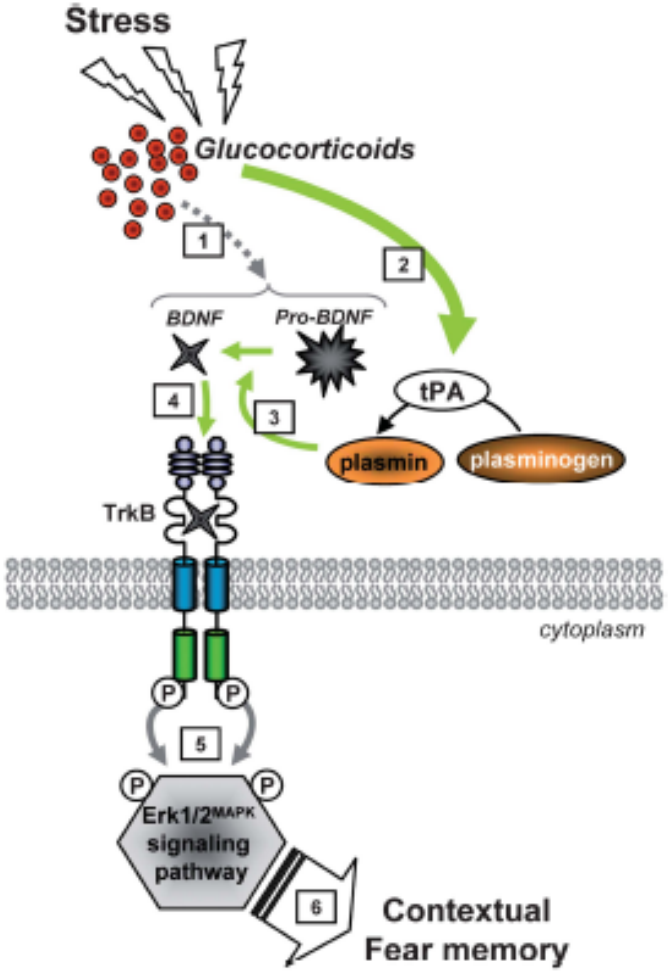
Sequestration in cytosol

Increased protein degradation

mRNA levels increased: Are putative/identified miRs altered?

GR and ERK1/2 role in fear conditioning





Revest et al. 2014
Mol Psychiatry

Revest 2014 Front. Psychitary

ERK1/2

ERK1 and ERK2 are members of the extracellular signal-regulated kinase family of mitogen-activated protein kinases.

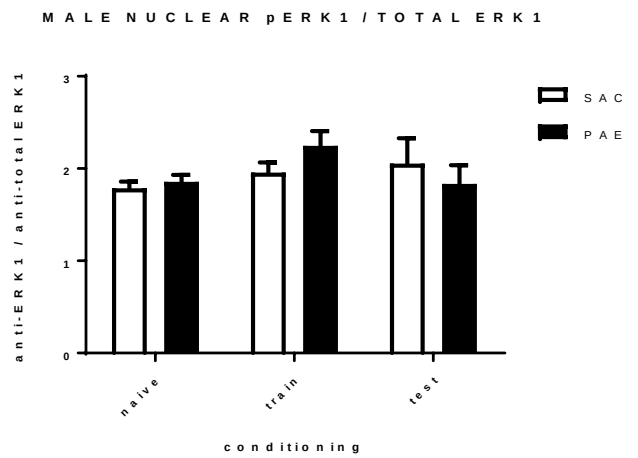
ERK2 plays an important role in fear-conditioned learning and memory, while ERK1 may not.

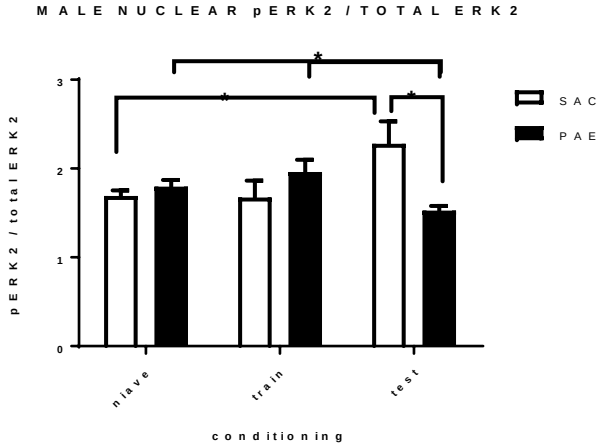
ERK1 and ERK2 reside in both the cytosol and nucleus. We focused our study on nuclear ER1 and ERK2.

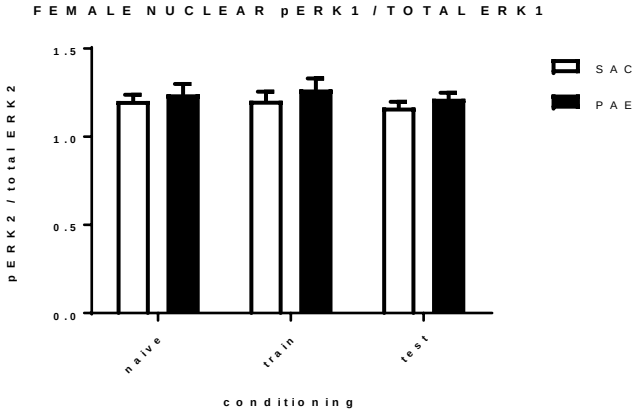
ERK1 and ERK2 catalytic activities are regulated by phosphorylation at two sites near the catalytic site: T183 and Y185 in ERK2.

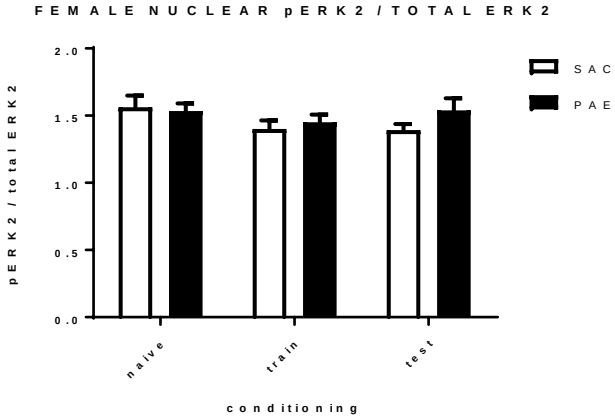
The activation state of ERK2 and ERK1 is commonly determined using immunoblotting techniques as the ratio of the anti-phospho-ERK1/2 immunoreactivity to the anti-total (phosphorylated and unphosphorylated) ERK1/2 immunoreactivity.

Nuclear ERK1/2: Nuclear ERK2 activation is increased following fear memory retrieval (testing) in SAC, but not PAE, males









Summary of biochemical studies

Males and females appear to differ in the biochemical mechanisms that underlie learning and memory formation.

The alterations in the GR and ERK2 pathways in PAE males are consistent with their playing a role in the contextual fear deficits in these animals.

The subcellular localization of the GR and the activation state of ERK1/2 are potential targets for therapeutic intervention in males.

The identification of therapeutic targets for females requires further study.

Relevance to human populations

Males and females learn differently.

PAE produces differing biochemical changes in males and females. Should we be treating males and females differently?

Is there a role for estrogen as a protective factor- *in utero* and/or during development?

Does the maternal and early-life social environment modify the observed behavioral and/or biochemical deficits?