KING'S College LONDON
Founded 1829

Models of Stress and Reproductive Dysfunction

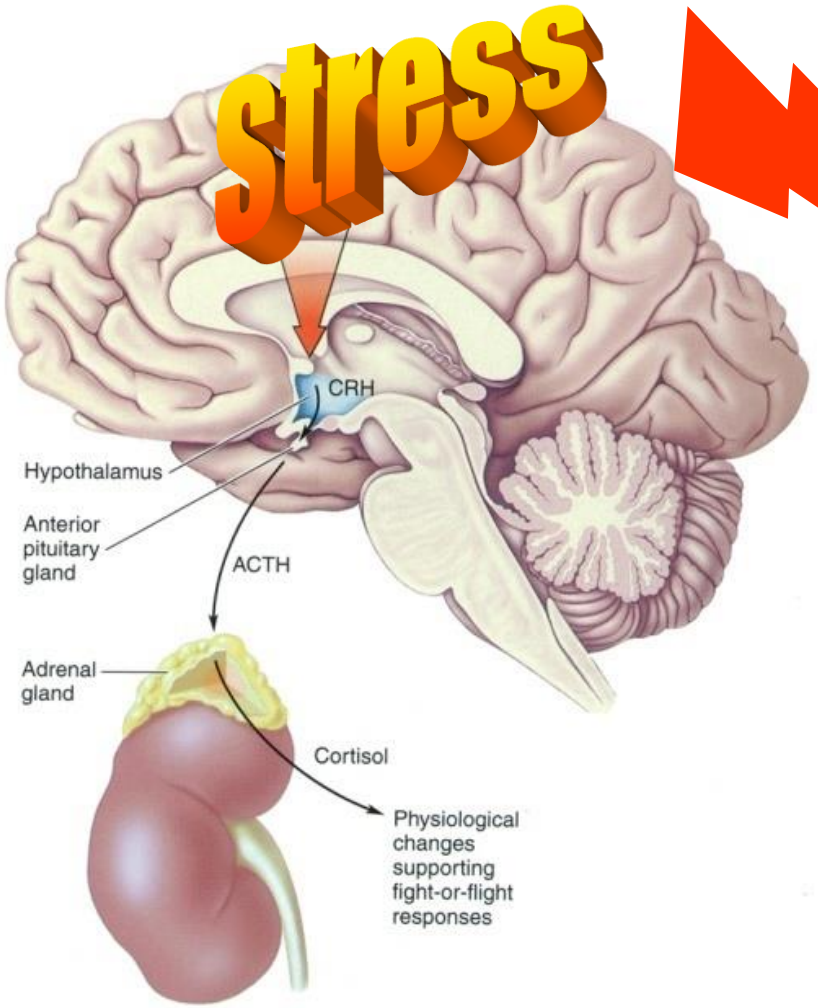
Workshop

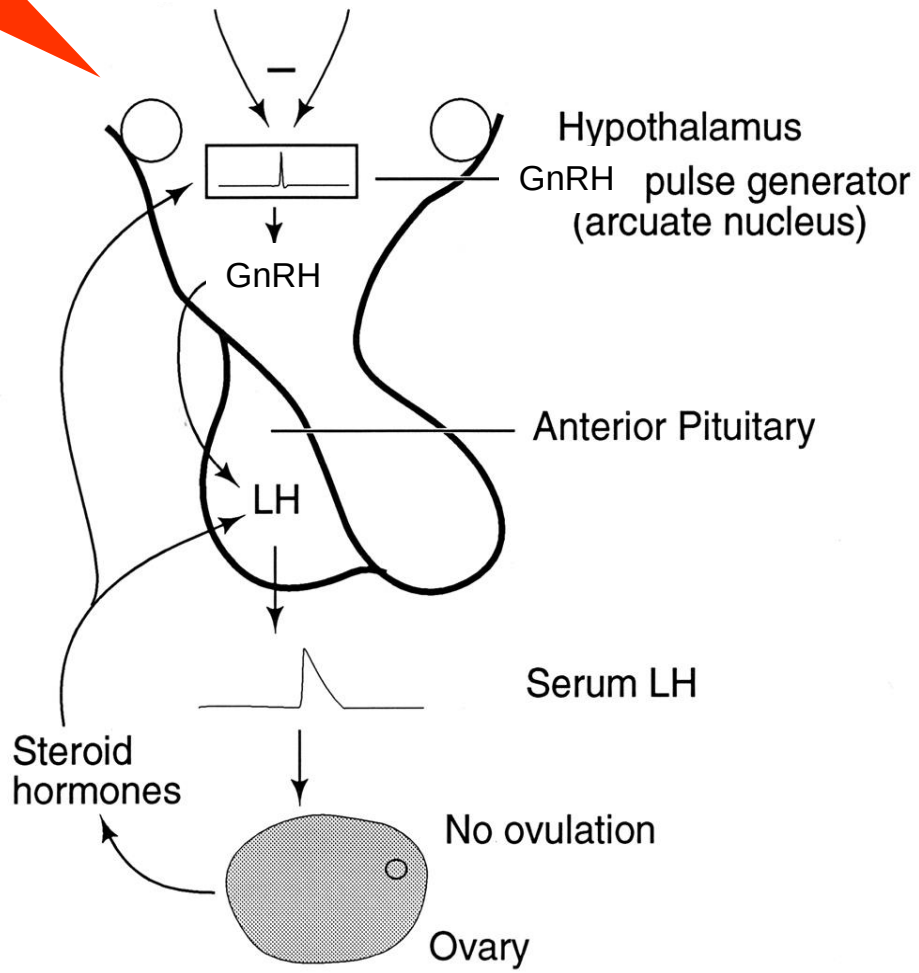
Kevin O'Byrne

Division of Endocrinology & Reproduction

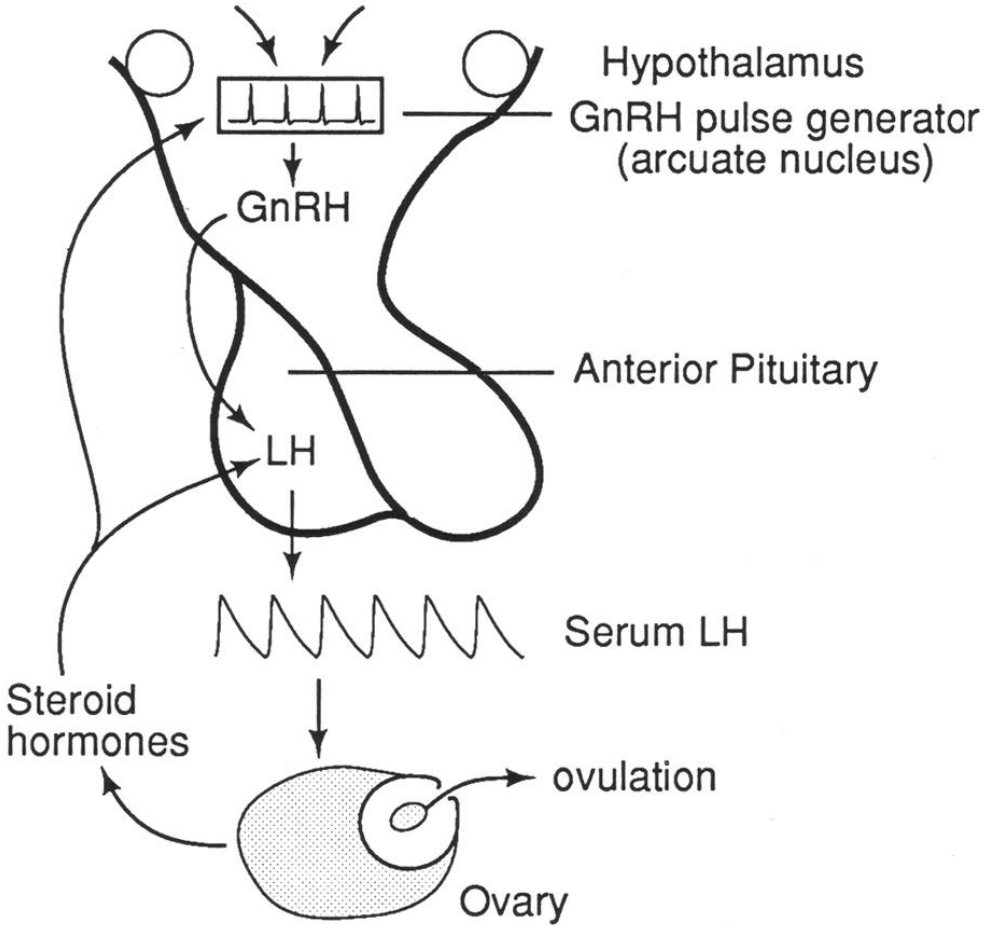
School of Biomedical & Health Sciences

Hypothalamic amenorrhoea





Monitor activity of GnRH pulse generator

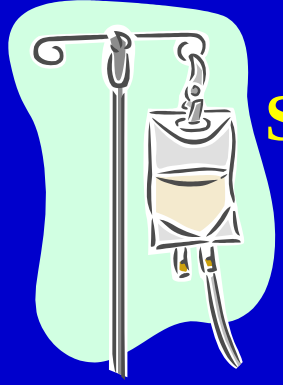


***<u>In vivo</u>*:**

- LH pulses
- GnRH pulses
  - Pituitary portal blood
  - Cerebrospinal fluid
  - Extracellular fluid (dialysis)
- MUA volleys

***<u>In vitro</u>*:**

- GFP-identified GnRH
- Primary cultures or GT1-7
  - Electrical activity
  - Calcium oscillations
  - GnRH pulses



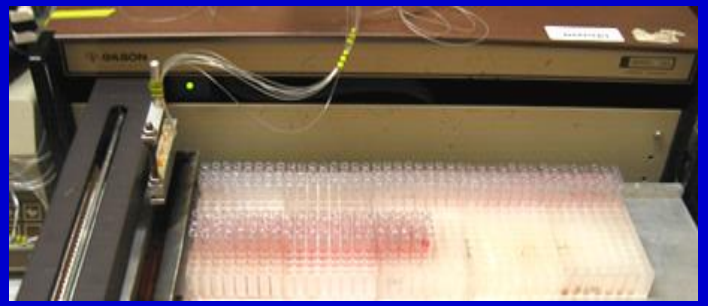
SALINE
HEPARIN

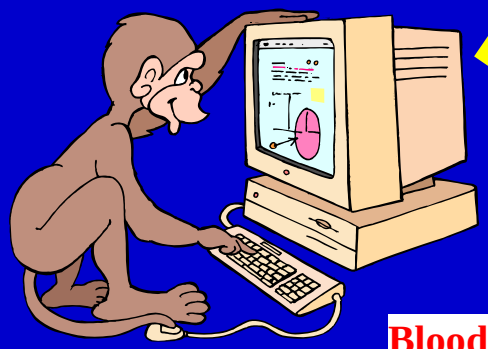
PUMP

Dual channel swivel

3 way solenoid valve

25 µl at 5 min interval for 6-10 hours





Blood travels along the pathways marked in red

Monitor activity of GnRH pulse generator: Human

***In vivo*:**

- LH pulses ✓
- GnRH pulses
  - Pituitary portal blood
  - Cerebrospinal fluid
  - Extracellular fluid (dialysis)
- MUA volleys

***In vitro*:**

- GFP-identified GnRH
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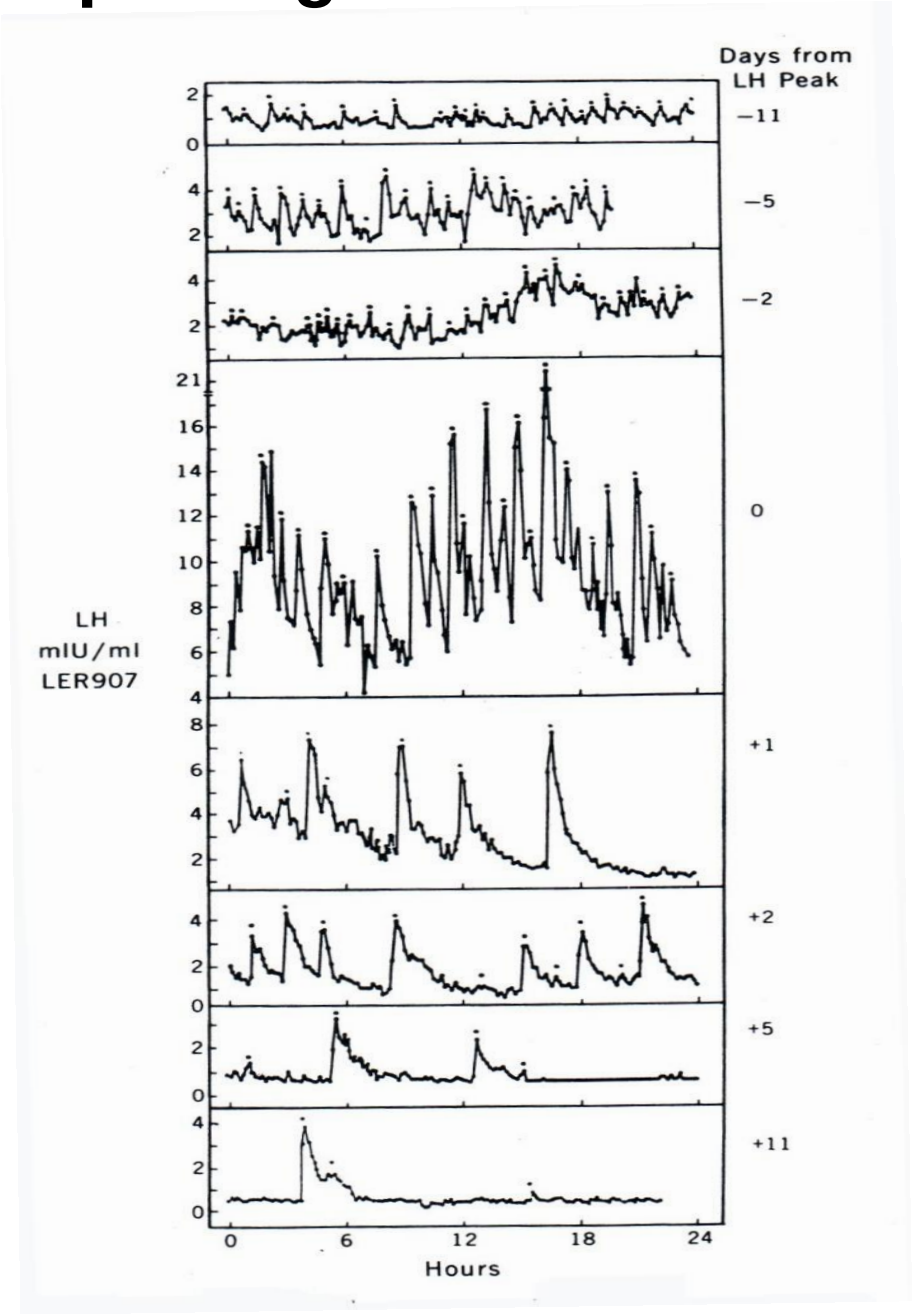
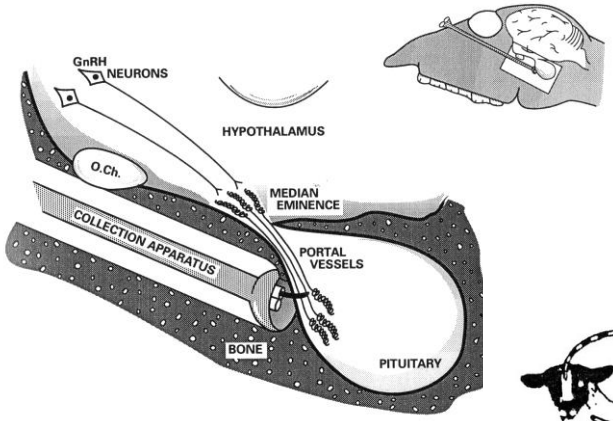
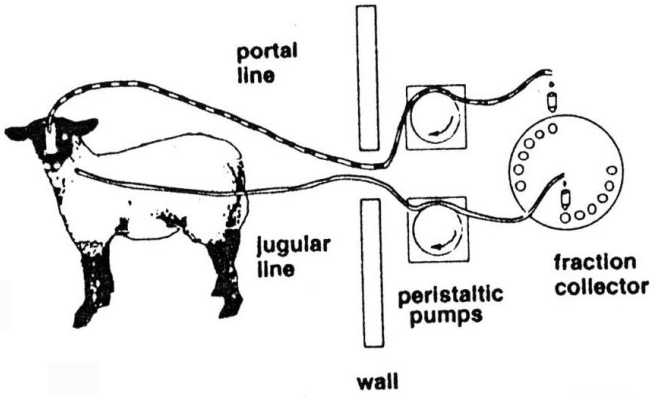
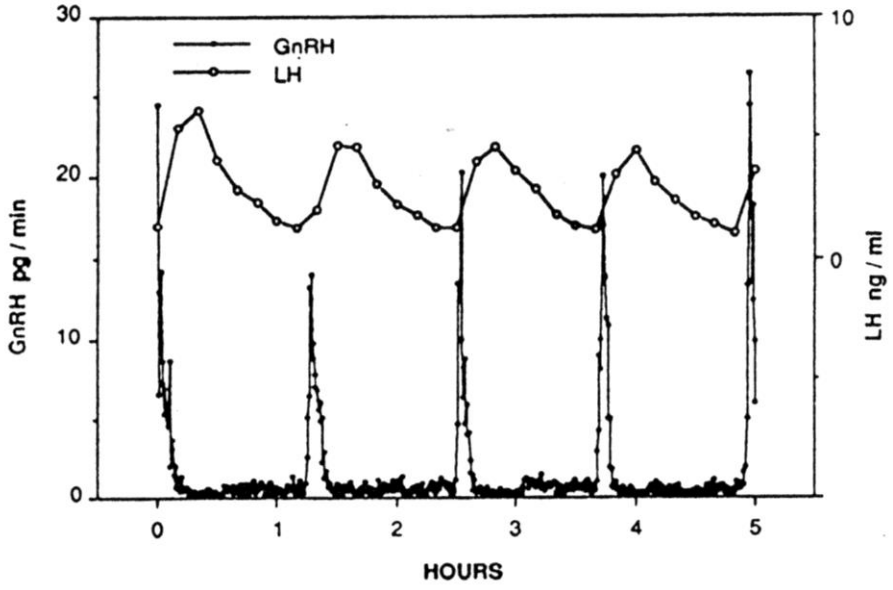


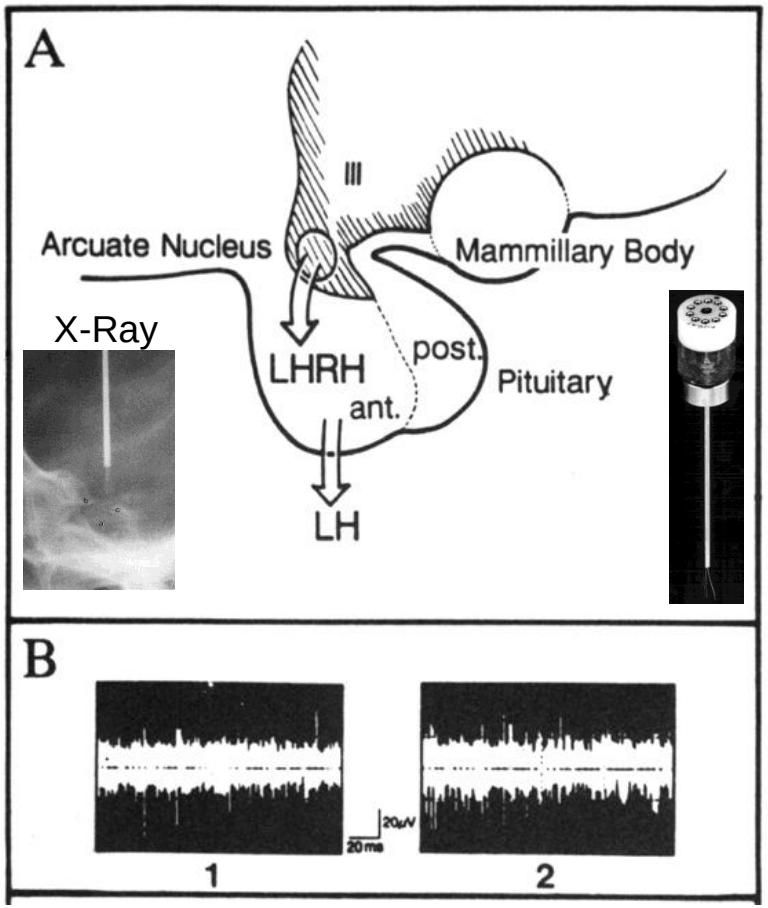
Monitor activity of GnRH pulse generator: GnRH Pulse

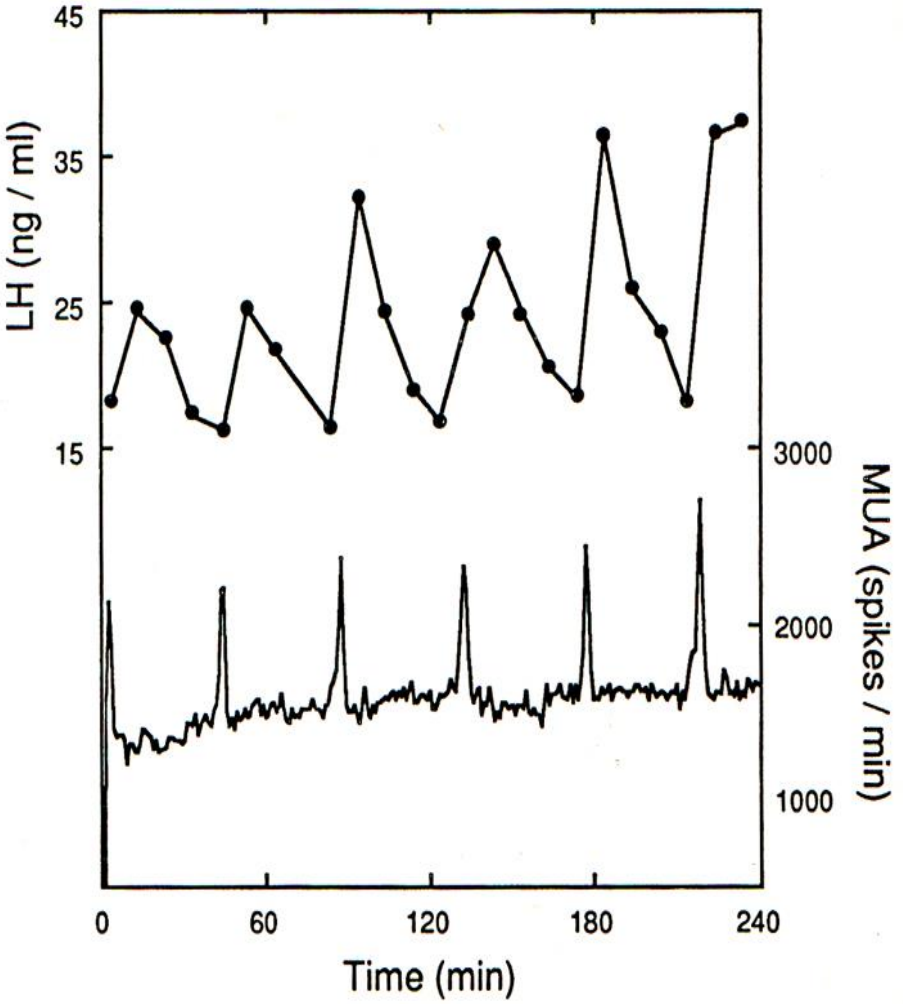




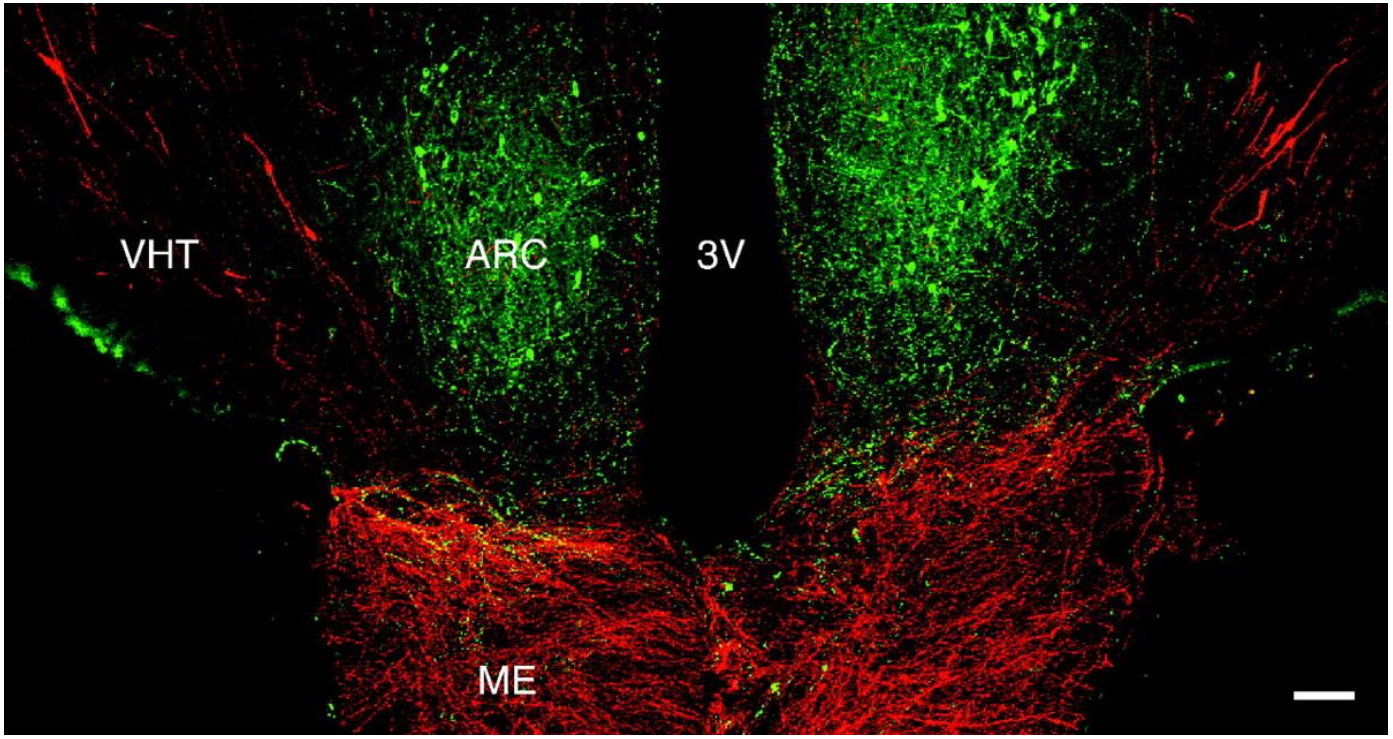


Monitor activity of GnRH pulse generator: MUA Volleys

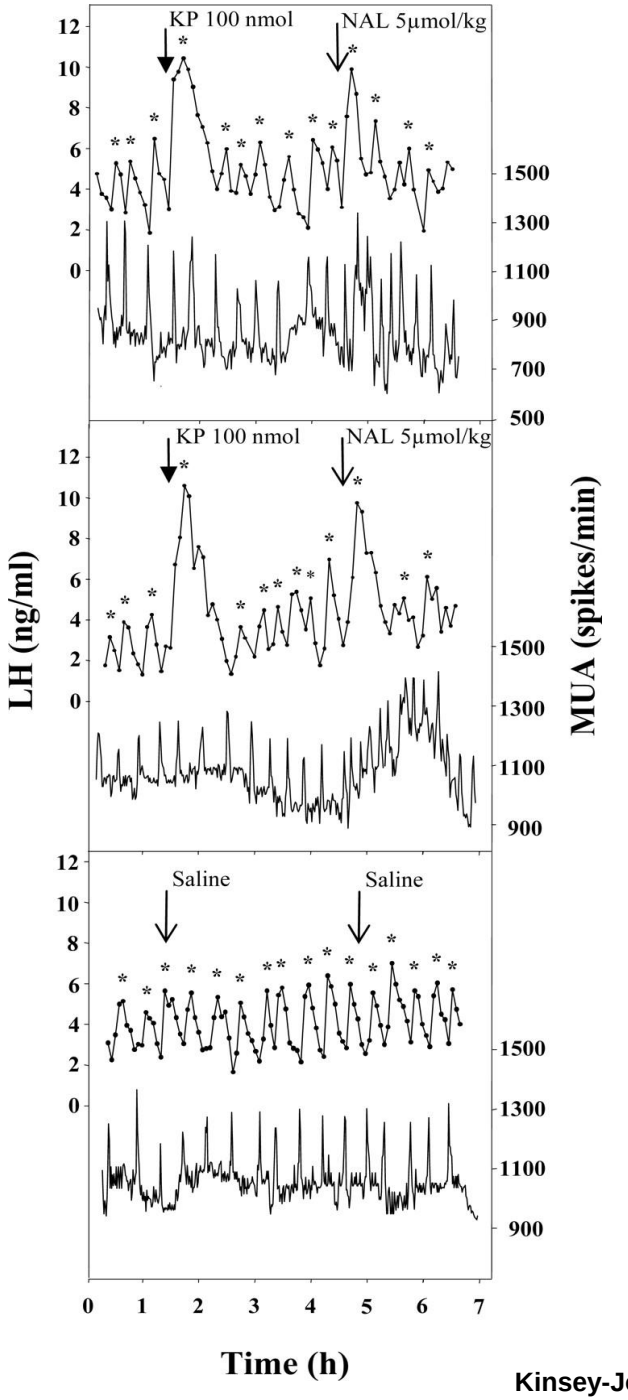




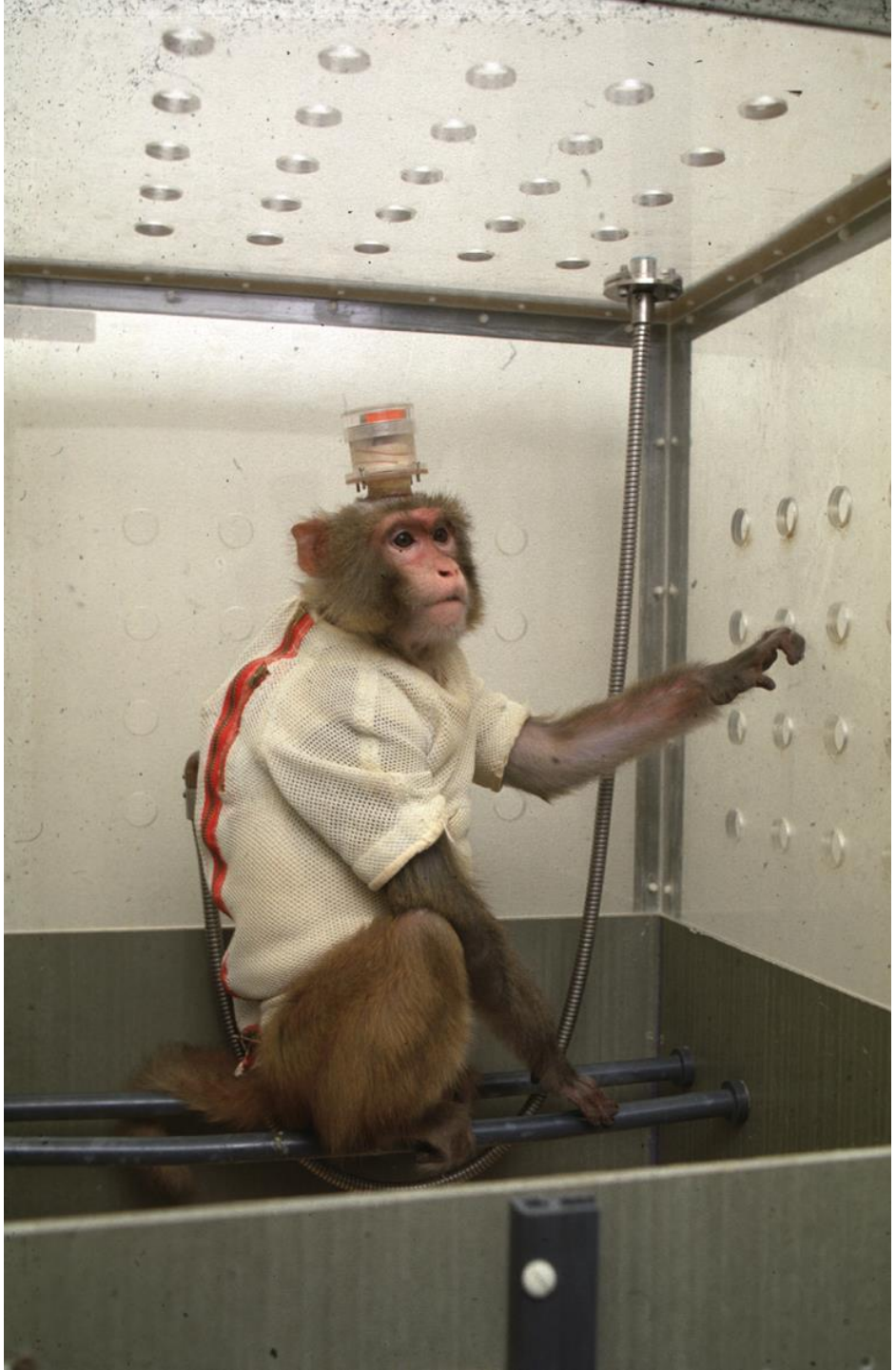
Kisspeptin (green) and GnRH (red) neurones in the male rhesus monkey

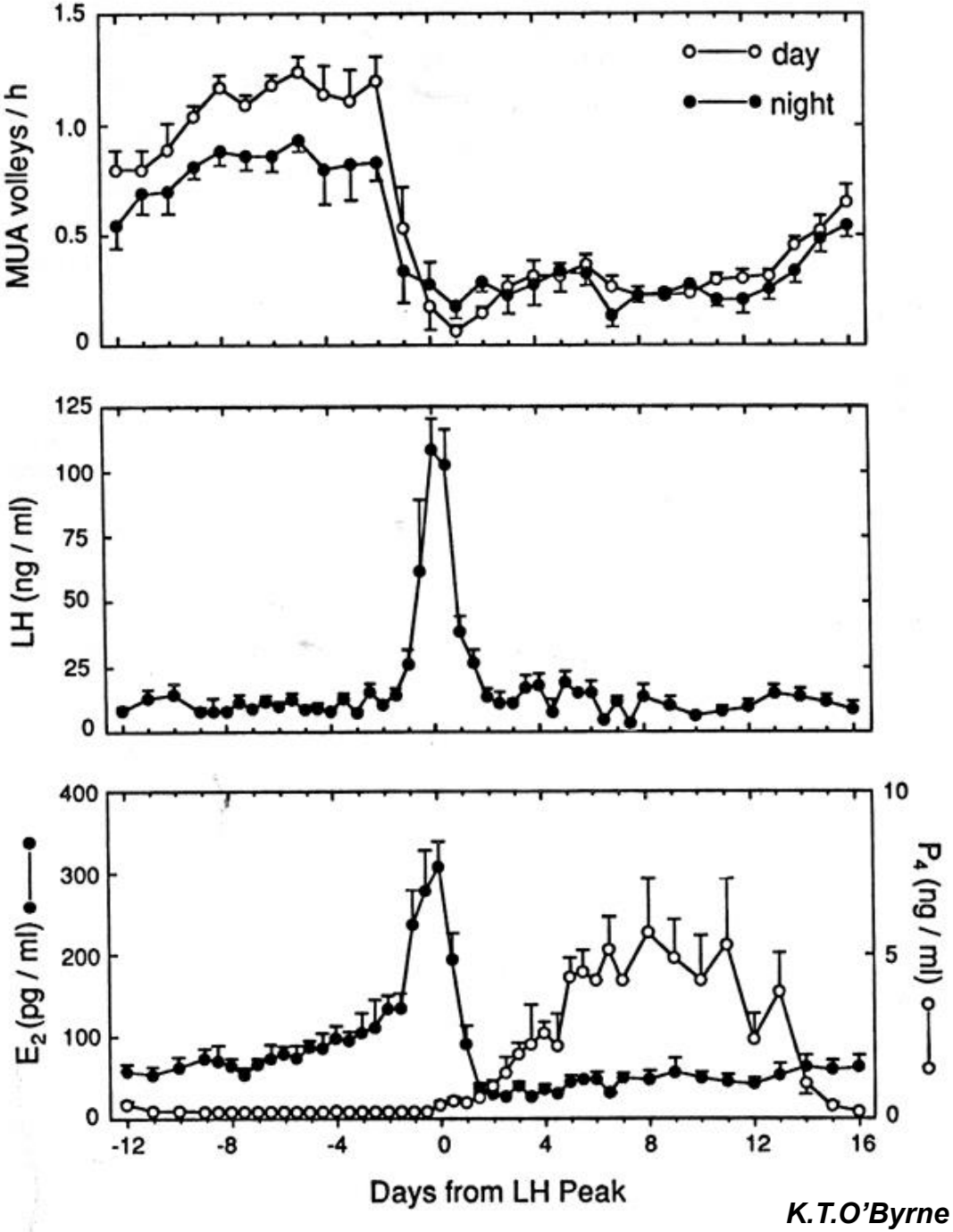


Ramaswamy, S. et al. Endocrinology 2008;149:4387-4395

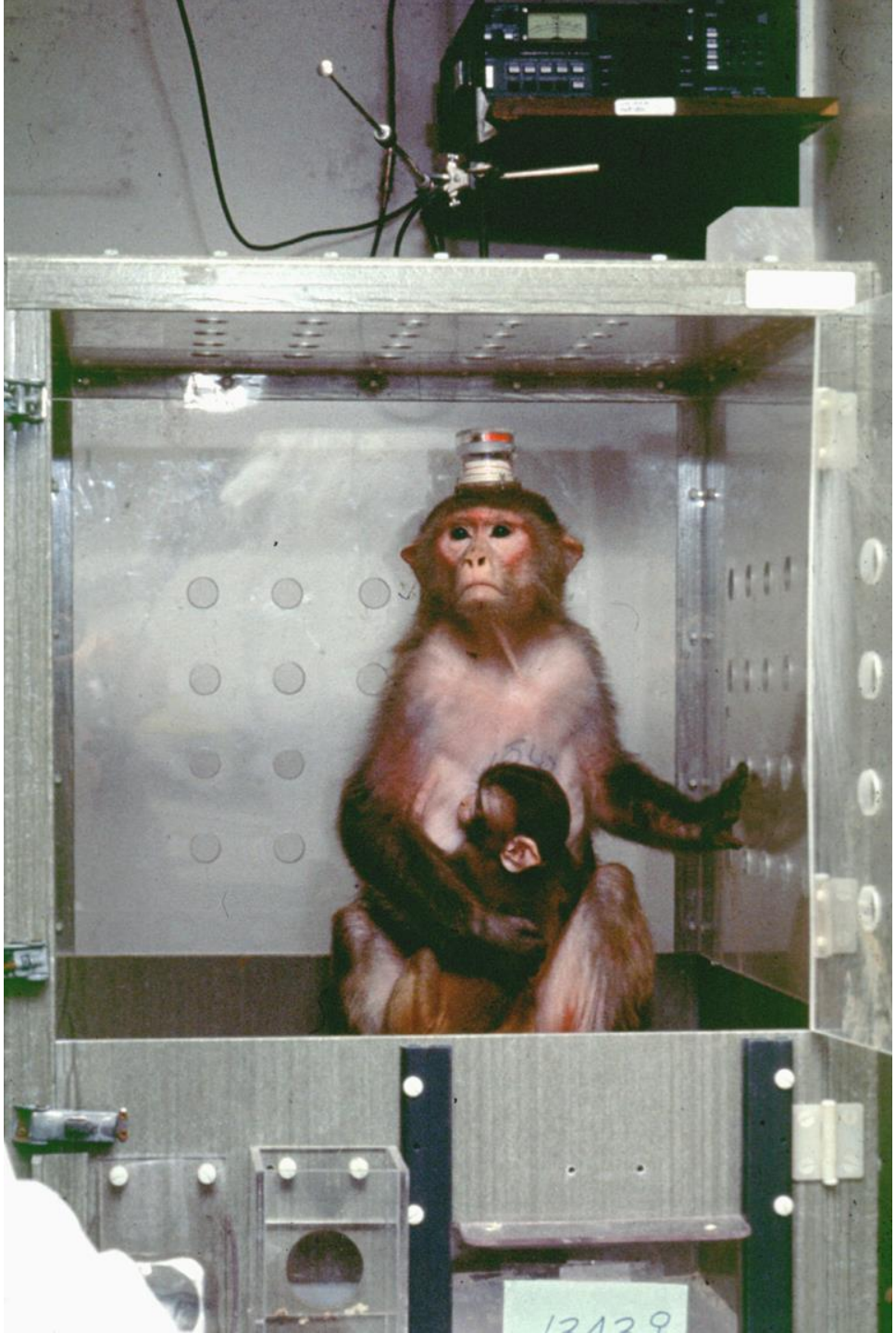


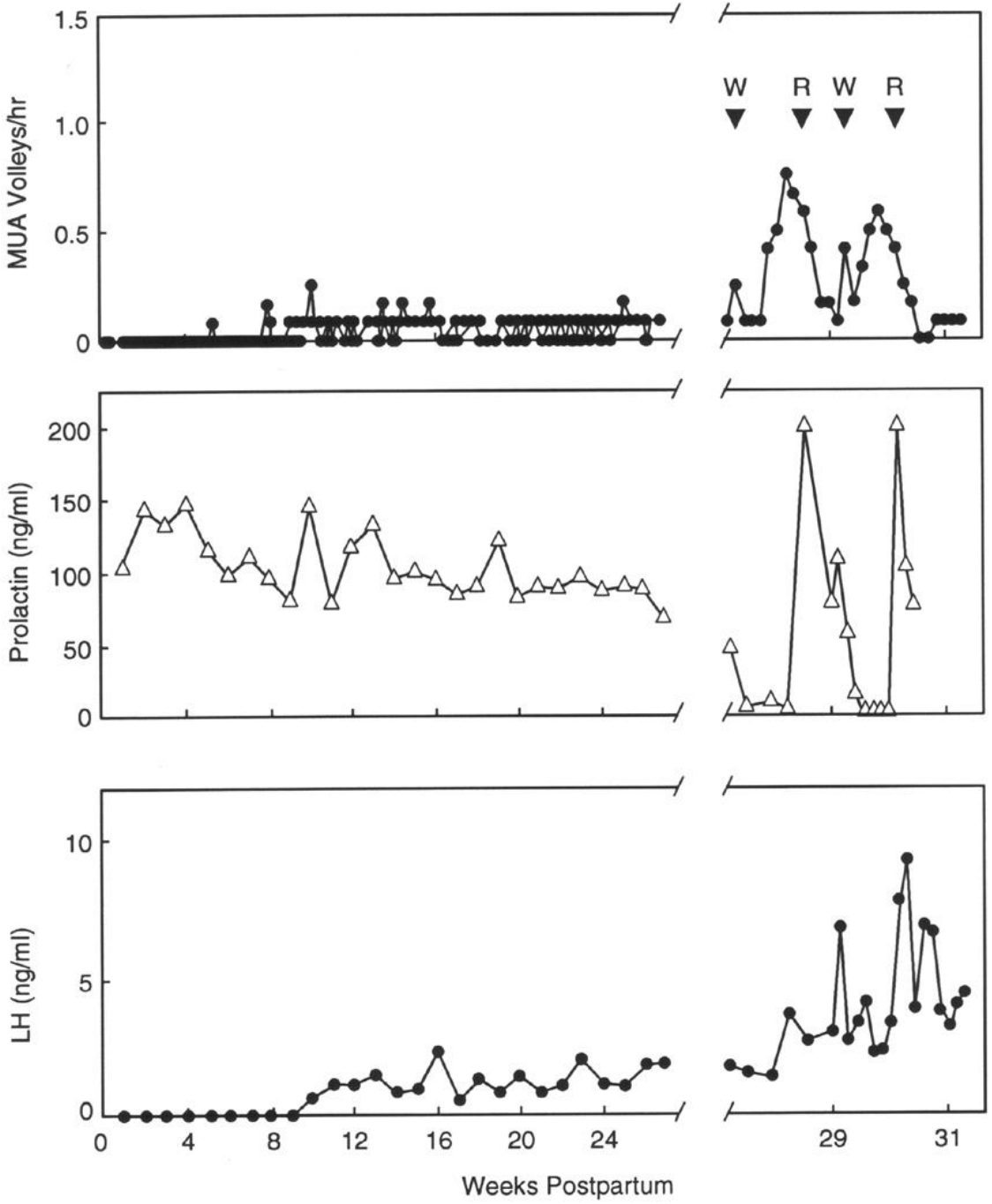
Kinsey-Jones, J. S. et al. Endocrinology 2008;149:1004-1008





K.T.O'Byrne et al, Endocrinology, 1991




MUA Volleys/hr
1.5
1.0
0.5
0
W
R
W
R
Prolactin (ng/ml)
200
150
100
50
0
LH (ng/ml)
10
5
0
0
4
8
12
16
20
24
29
31
Weeks Postpartum

Methodological strategies to manipulate the HPG axis

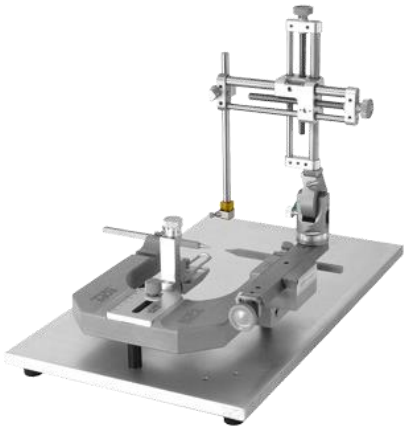
Pharmacological tools

- Classical stress neuropeptides and their antagonists:
  - CRF, Vasopressin, Opioids, CGRP, etc.
- Myriad other modulators:
  - Cytokines, prostaglandins, etc.

Routes of administration

- Peripheral:
  - Iv, ip, sc, etc.
- Central:
  - Intracerebroventricular
  - Intra-nuclear (unilateral vs bilateral)

Osmotic mini-pump – prepubertal rats



Methods:

- Post natal day 28 female rat (~65 g)
- 28 gauge icv cannula (Plastics One)
- Alzet mini-pump (~1g) (0.5µl/h for a 14-day)
- CRF (400 pmol/day) or CRF antagonist (4 nmol/day)
- Controls: Non-surgical or aCSF
- Vaginal opening and First Oestrus (Puberty markers)

Results: Time of puberty

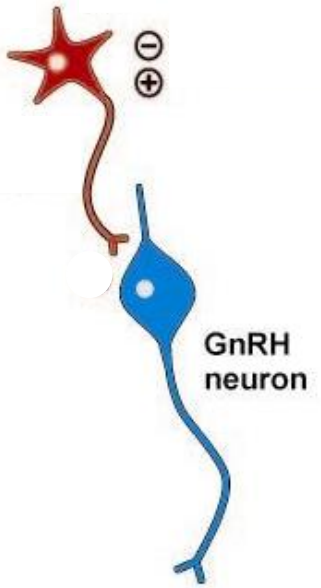
•Non-surgical = aCSF controls:	pnd 37
•CRF:	pnd 40
•CRF antagonist:	pnd 35

- (No change in body weight)

Problem: hierarchical control and integrative neurobiology?

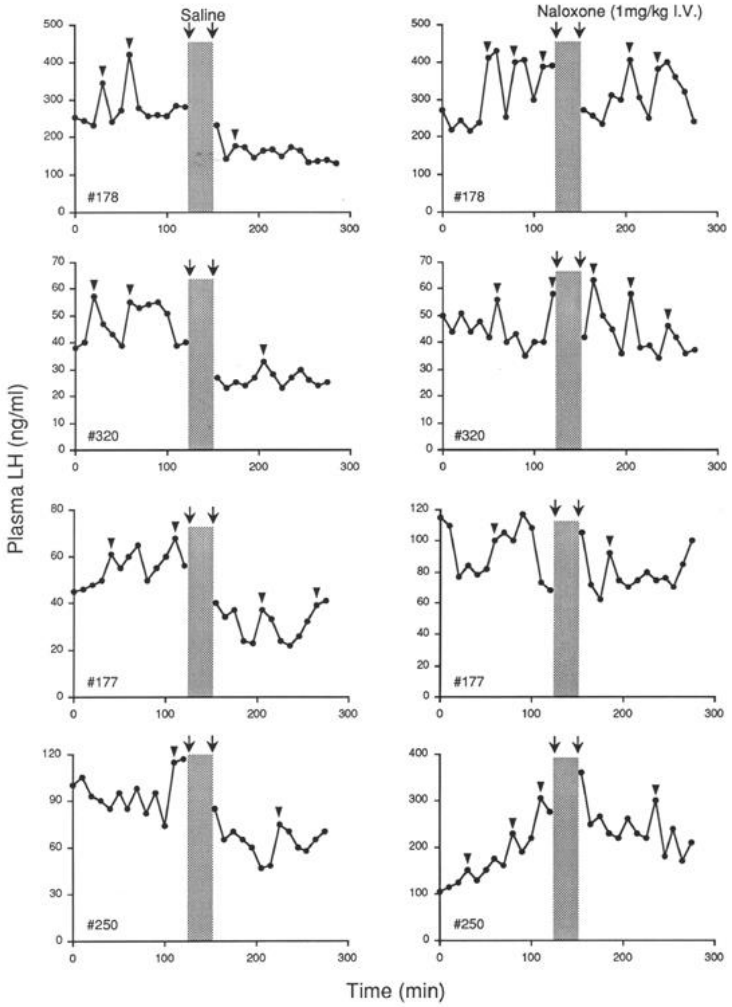
Neuropeptide synapses

5-HT
DA
NA
GnRH
CRF
VP
GAL
NT
SP
NPY
Kiss1
POMC
GABA
Etc.



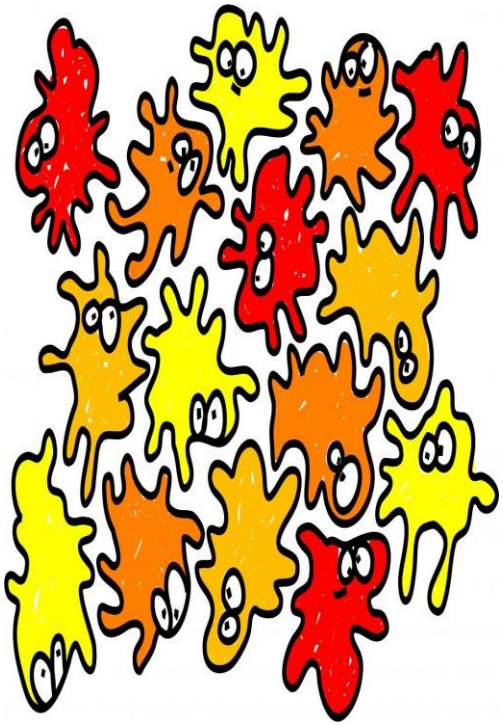
Physiological stressors?

Socially mediated infertility

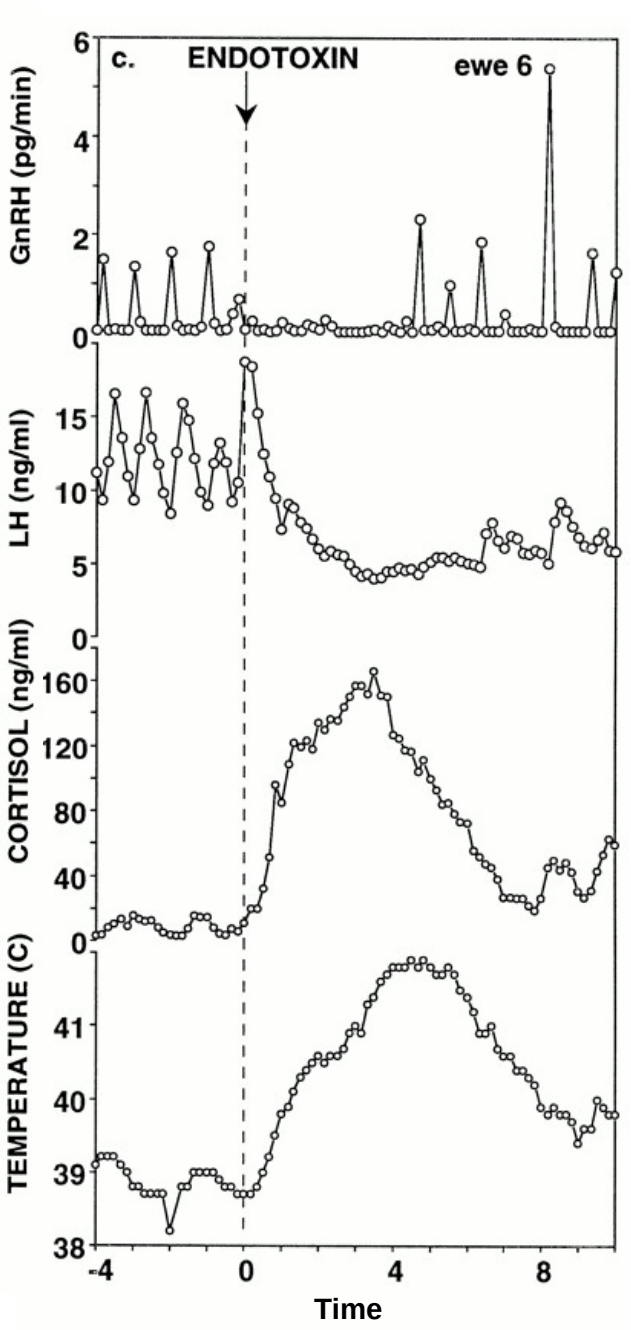


O'Byrne KT et al. Physiol Behav. 1989

Immunological stress



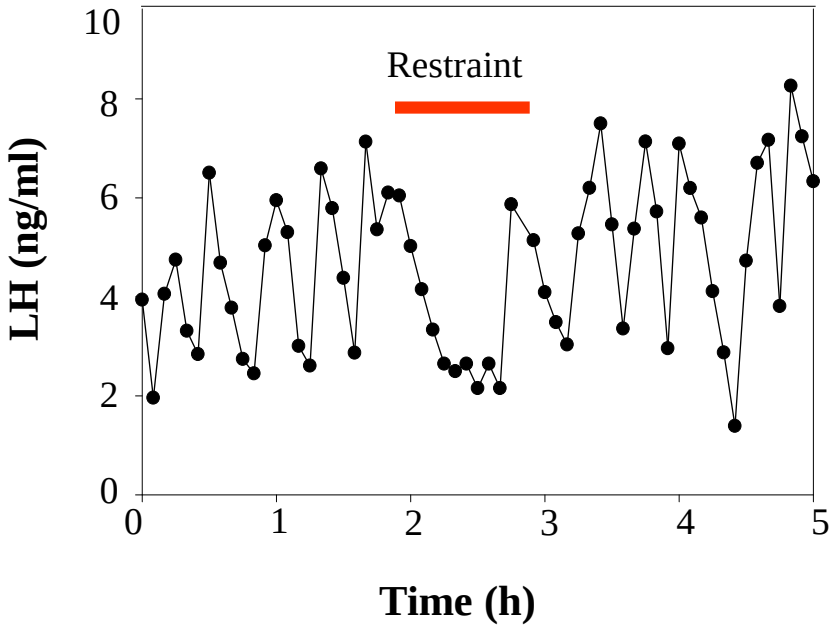
Endotoxin - LPS



Battaglia, D. F. et al. Endocrinology 1997

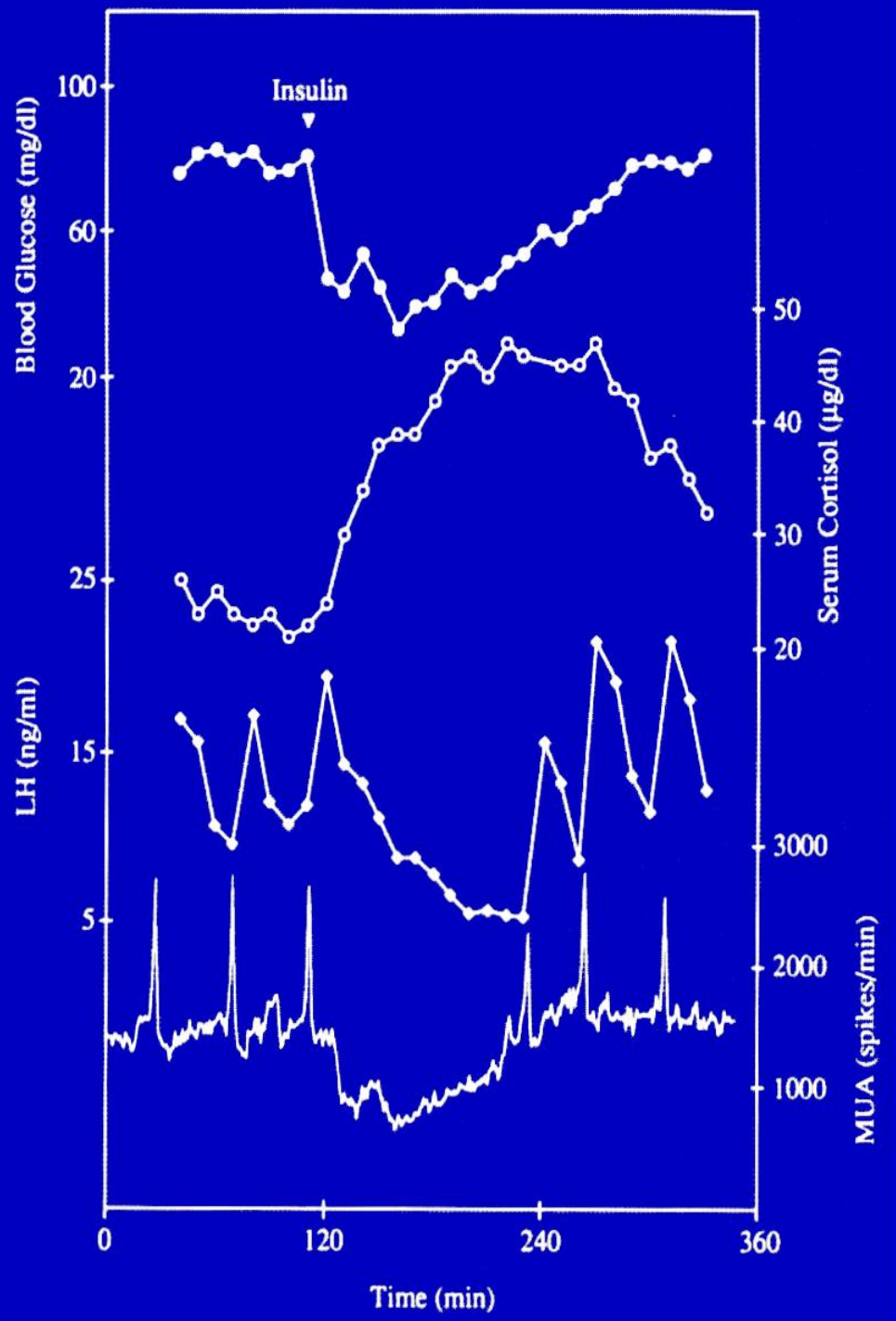
Restraint stress

I thought I'd take the tube to avoid the congestion charge



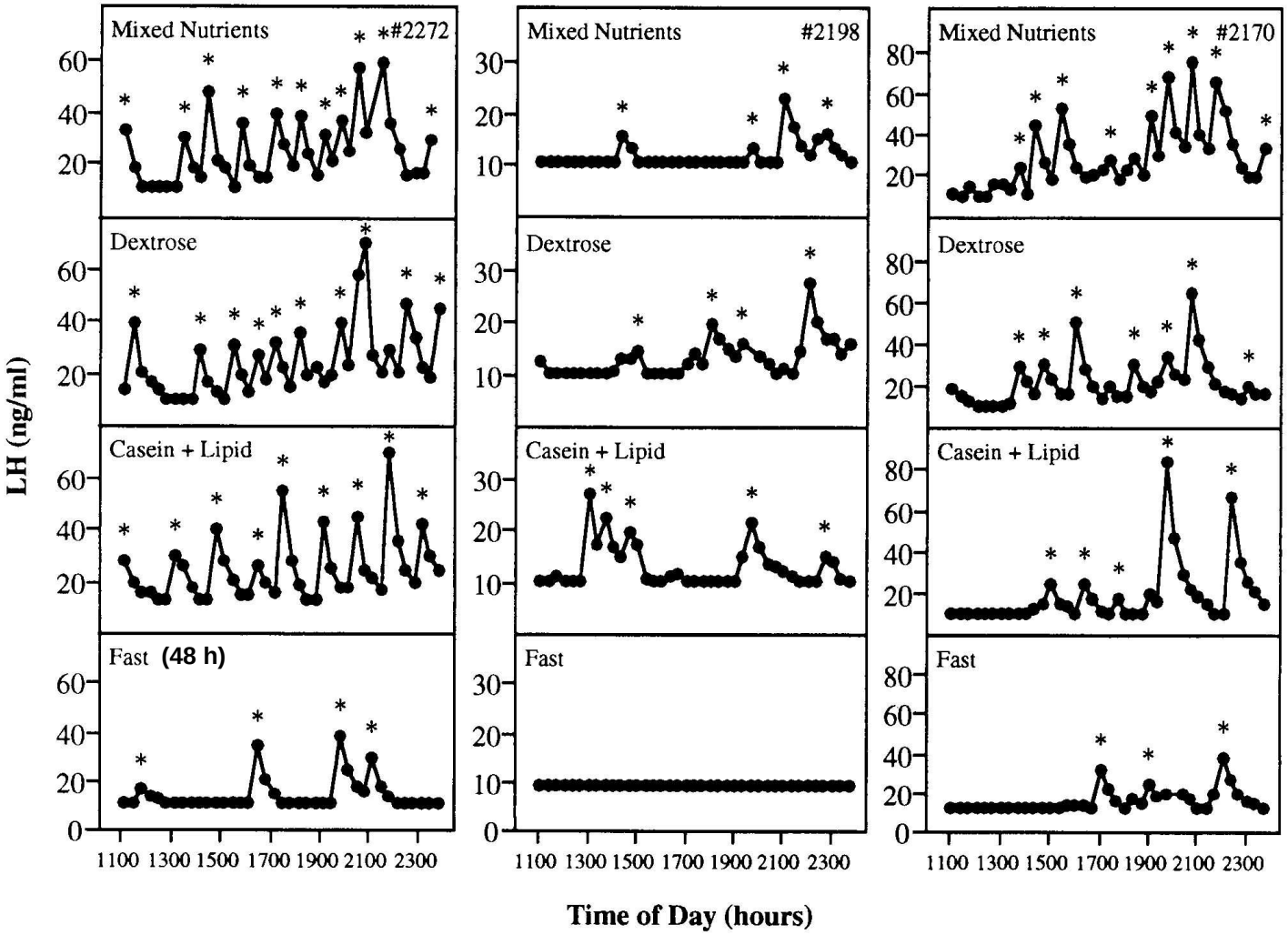
Restraint 1 hour

Hypoglycemia insulin iv



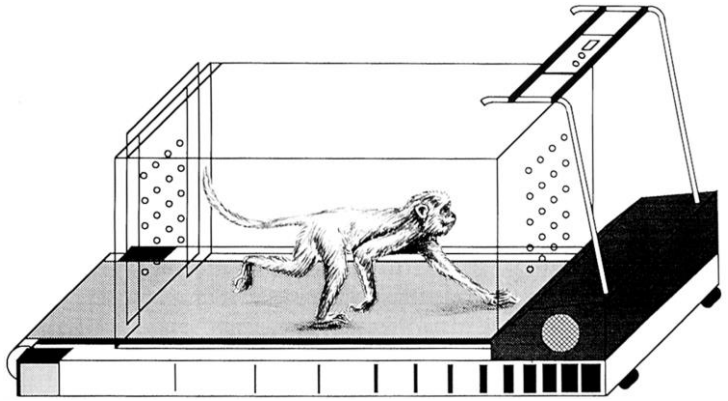
Chen et al. Neuroendocrinology, 1992

Fasting and re-feeding affects on pulsatile LH secretion

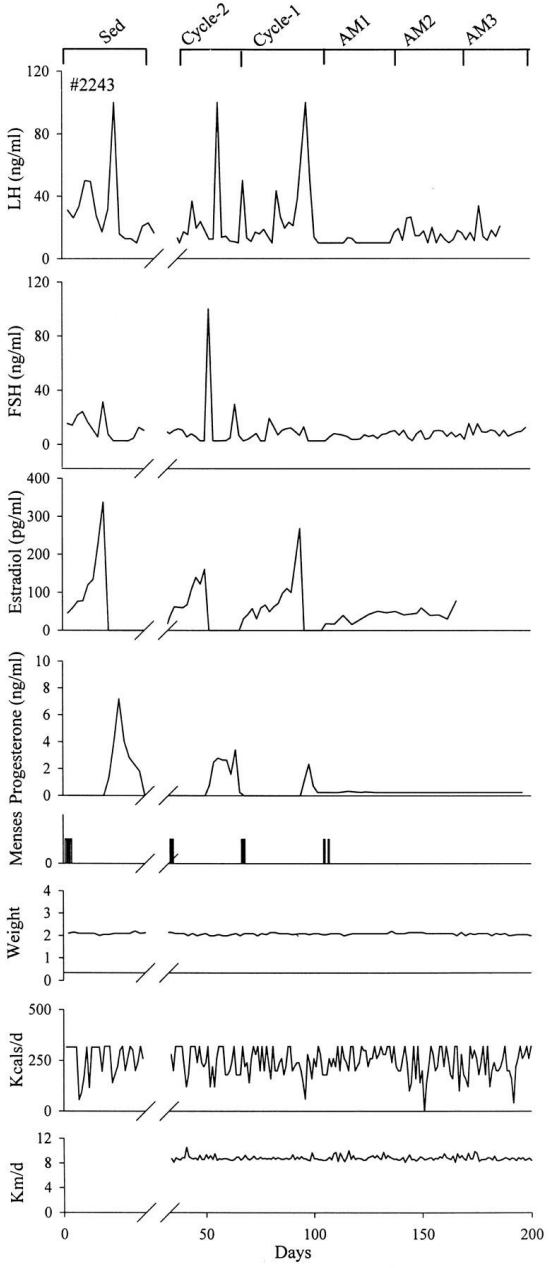


Schreihofer DA et al., Endocrinology 1996, 137:3770

Exercise-induced amenorrhoea



Cynomolgus monkey



Williams, N. I. et al. Endocrinology 2001

Clinically recognised forms of stress-induced reproductive dysfunction:

- Functional hypothalamic amenorrhoea:- psychosocial
- Anorexia (bulimic) nervosa:- nutritional compromise
- Exercise-associated:- excessive exercise

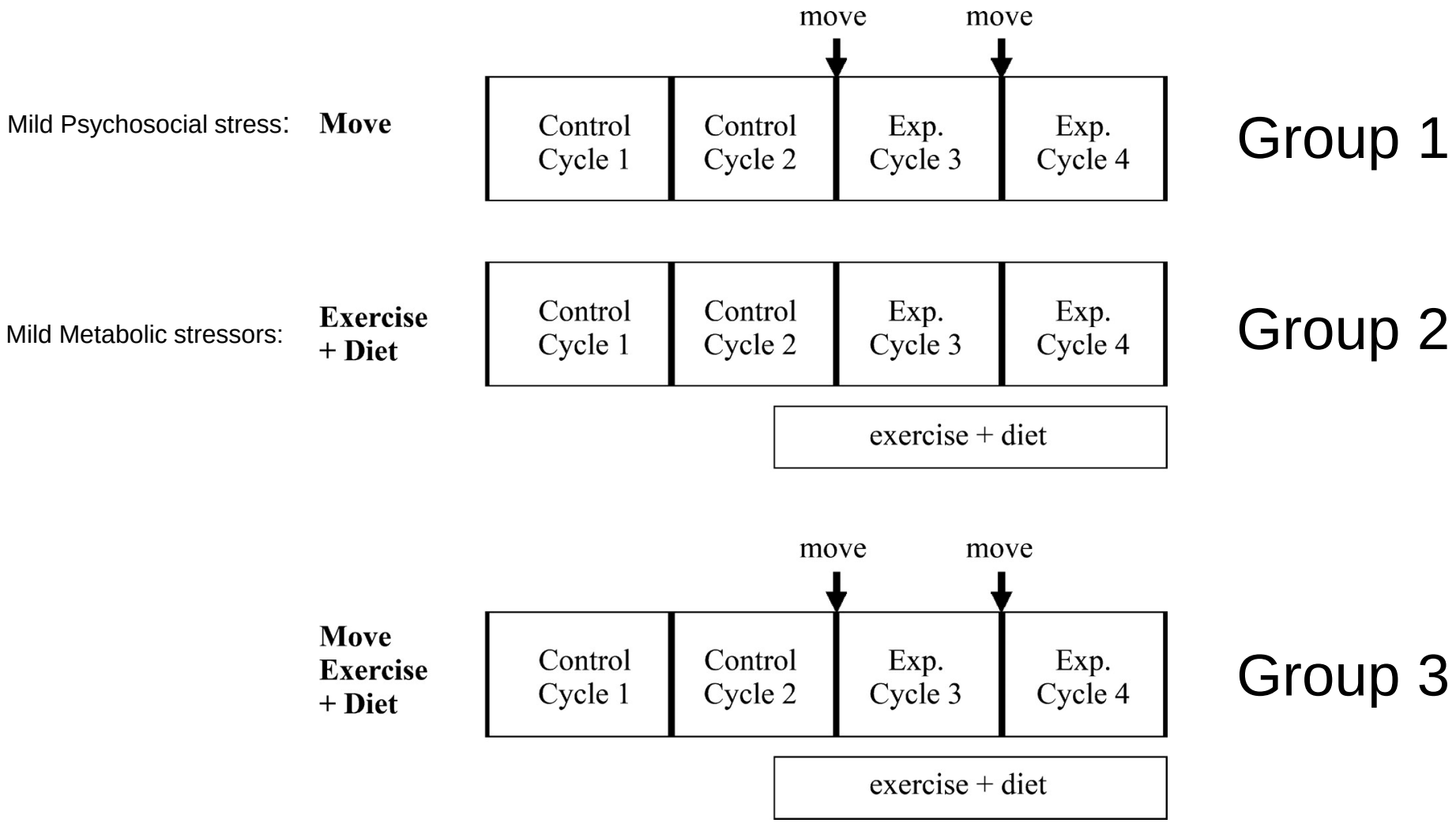
Growing recognition that each of these syndromes develops in response to exposure to combinations of psychogenic and metabolic stressors

Functional hypothalamic amenorrhoea

- *Mild indices of "psychological stress"*
  - Dysfunctional attitudes
  - Unrealistic expectations
  - Higher levels of perfectionism
  - Higher need for social approval
  - Difficulty coping with daily hassles

- *Mild metabolic deficits*
  - Higher incidence of subclinical eating abnormalities
  - exercising

Schematic diagram of the experimental design (monkey model of FHA)

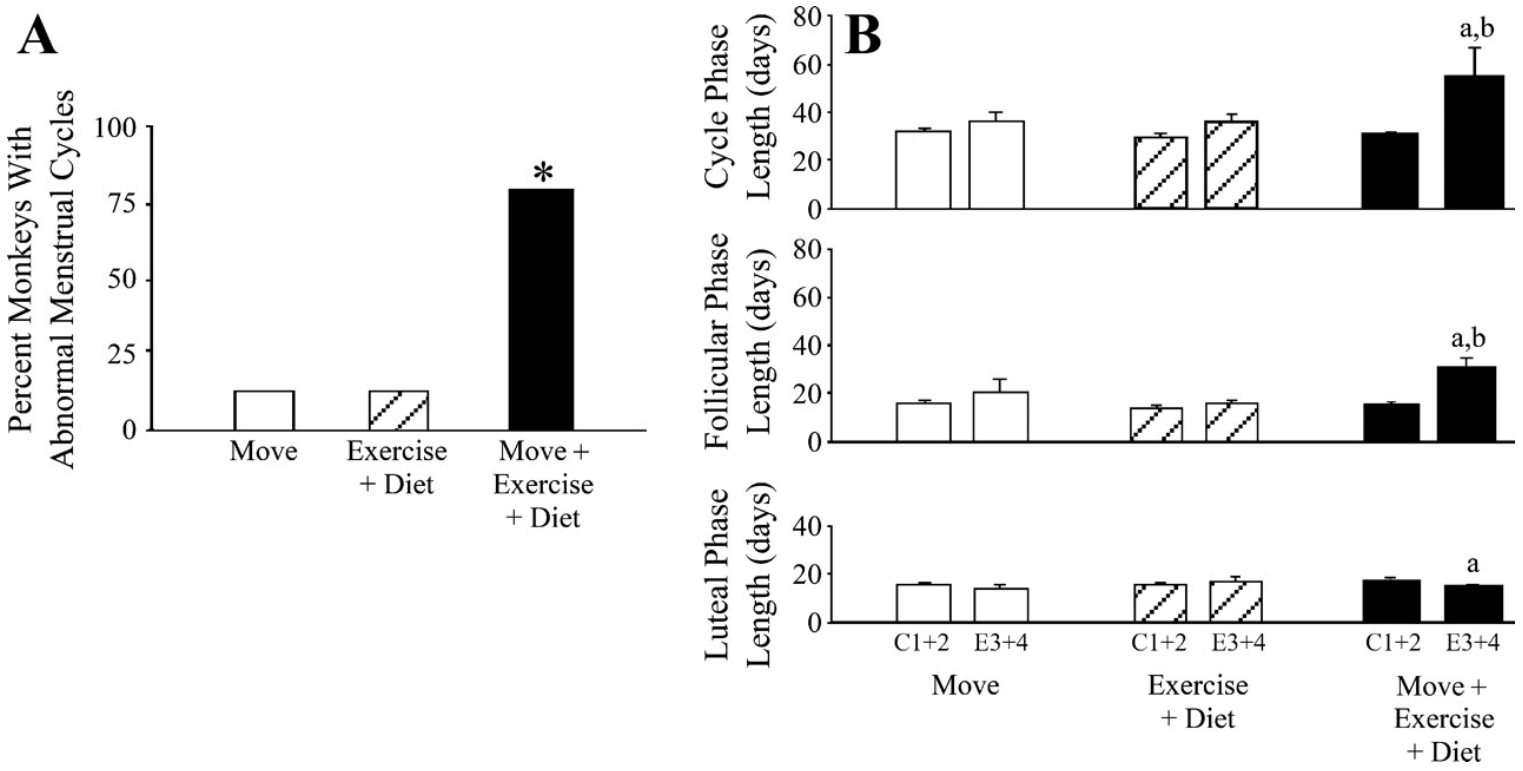


Williams, N. I. et al. Am J Physiol Endocrinol Metab 293: E270-E276 2007

AJP - Endocrinology and Metabolism

Percentage of monkeys showing abnormal menstrual cycles (i.e., either cycles >44 days in length or anovulatory cycles) in E3+4 for each of the 3 experimental groups

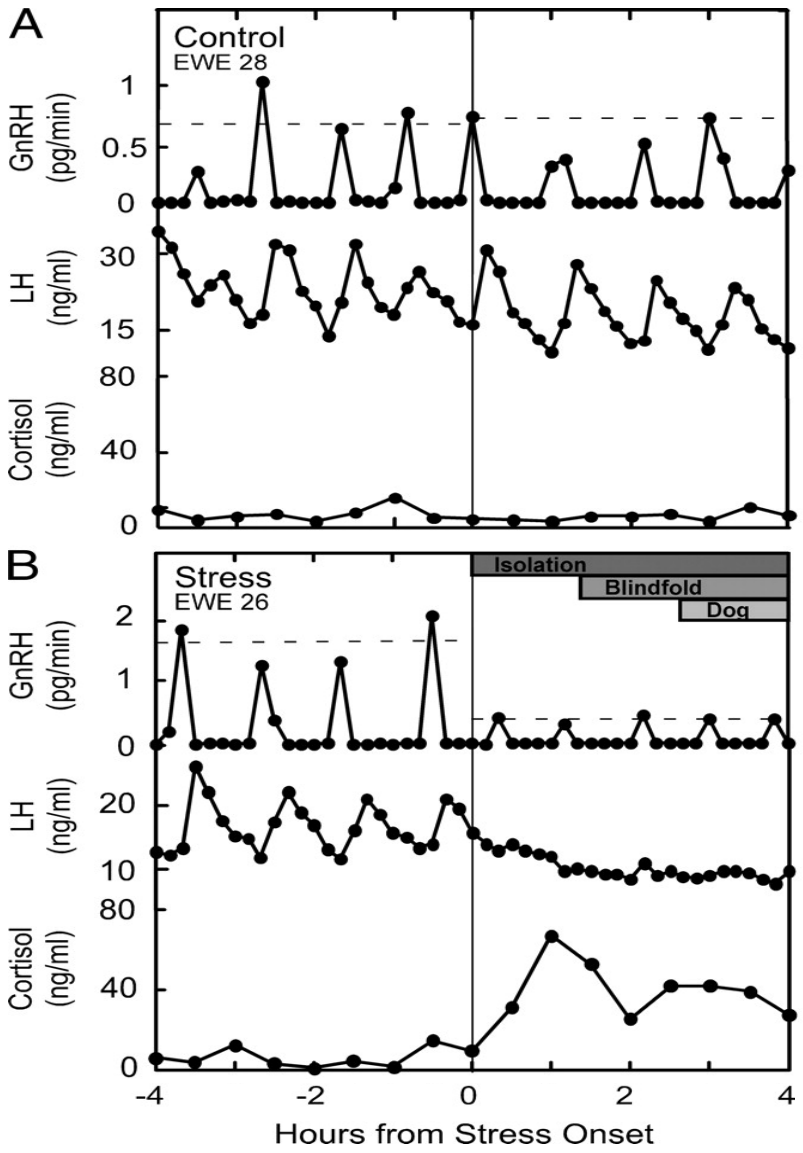
[Subthreshold stressors synergise to compromise reproductive function]



Williams, N. I. et al. Am J Physiol Endocrinol Metab 293: E270-E276 2007

AJP - Endocrinology and Metabolism

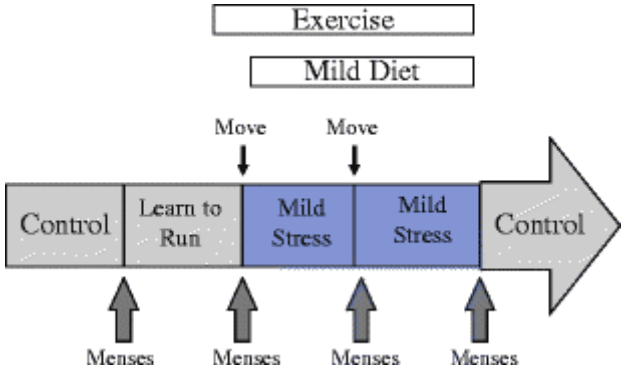
Effects of a layered stress paradigm on GnRH, LH & cortisol in the ewe

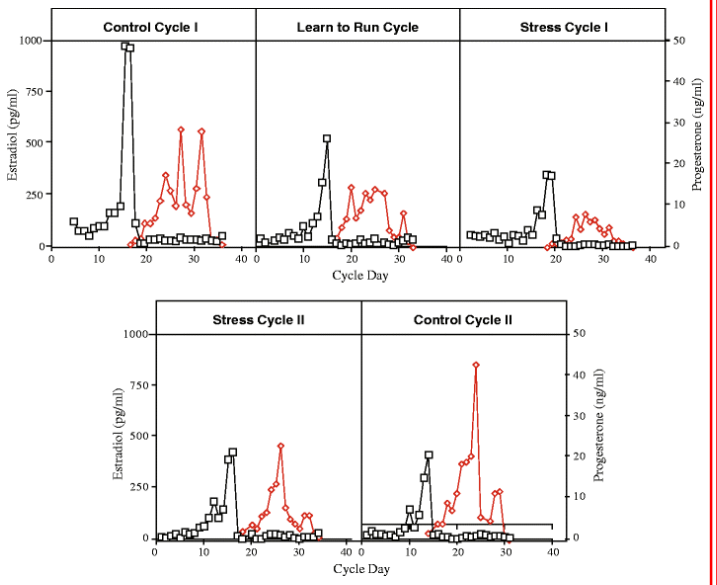


Wagenmaker, E. R. et al. Endocrinology 2009

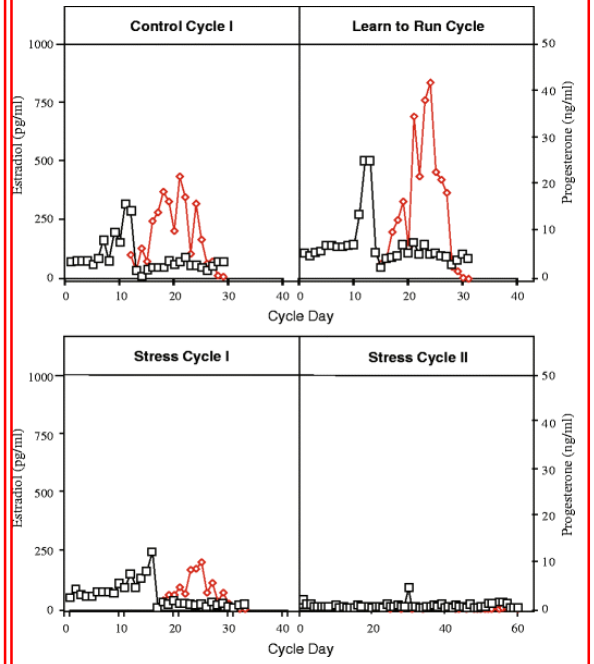
Individual stress-sensitivity & reproductive dysfunction in female macaques

Schematic diagram of experimental design

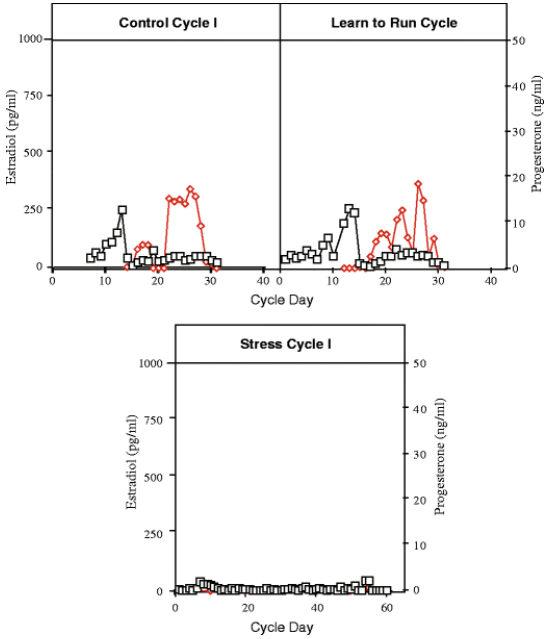




High stress resilience (HSR)



Medium stress resilience (MSR)



Stress sensitive (SS)

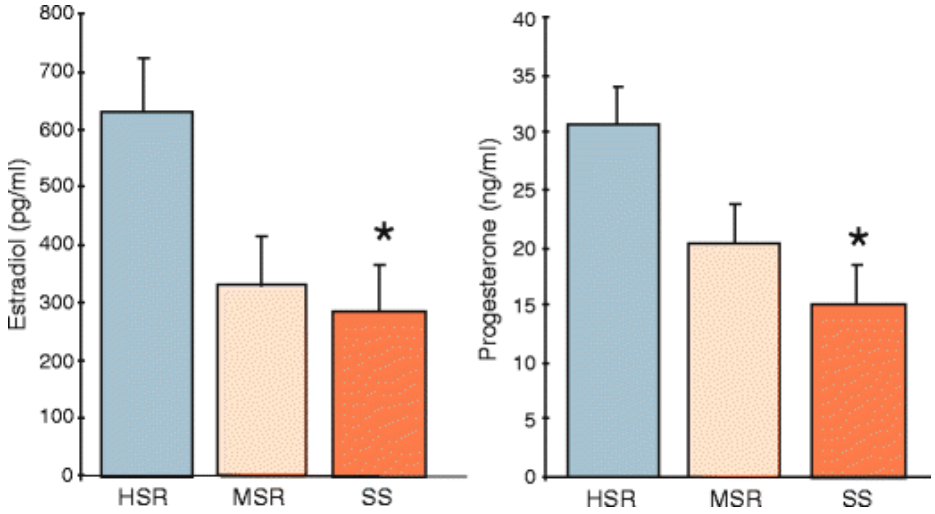
Bethea CL et al., Mol Neurobiol 2008 38:199

An amazing array of models of stress-induced reproductive dysfunction

Molecular tools?

- In Situ
- qRT-PCT
- Transgenics
- Etc.

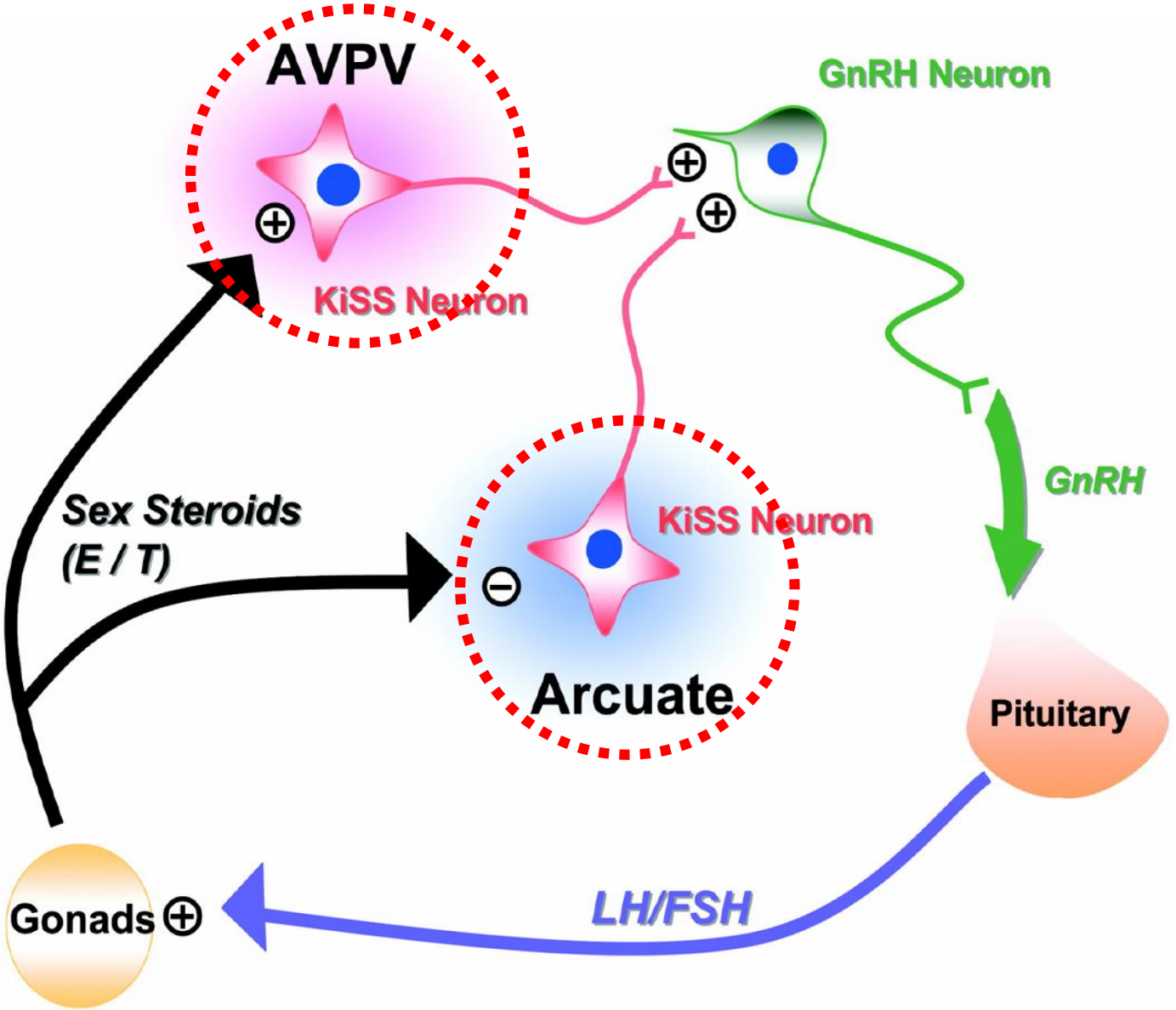
Cynomolgus monkeys: In the absence of stress



GnRH expression – hypothalamus	HSR>MSR>SS
Serotonin gene expression - Raphae:	HSR>MSR>SS
Number of serotonin neurones - Raphae:	HSR>MSR>SS
GABA expression – hypothalamus	SS>MSR>HSR
CRH expression – hypothalamus/amygdala	SS>MSR>HSR

Bethea CL et al., Mol Neurobiol 2008 38:199

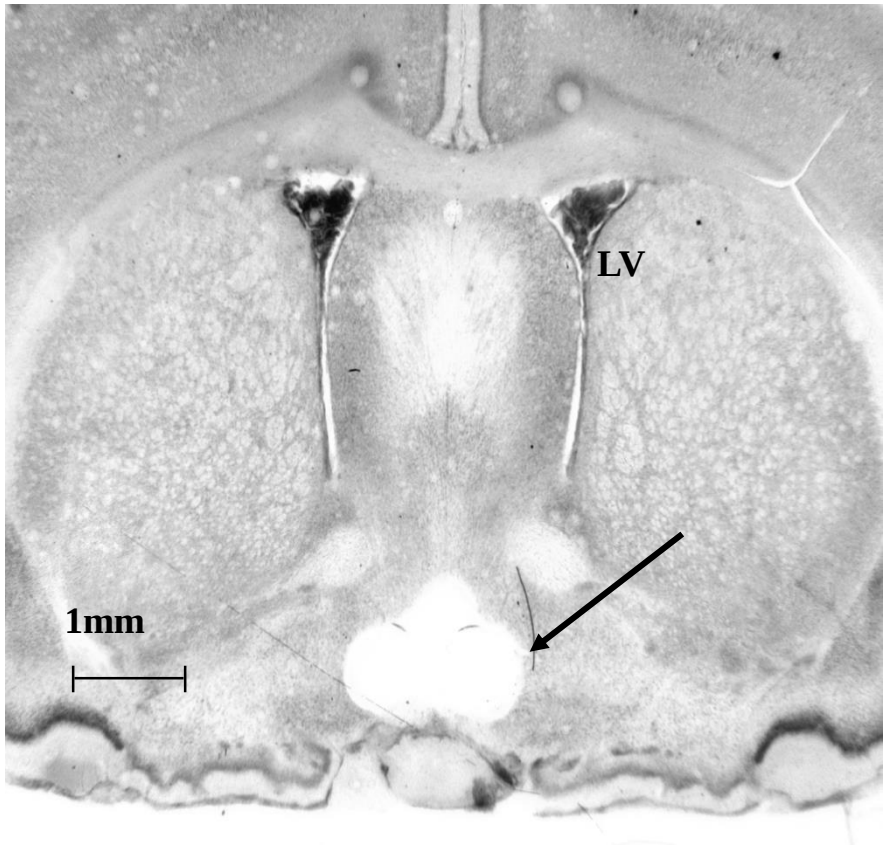
Kisspeptin stimulates the neuroendocrine reproductive axis



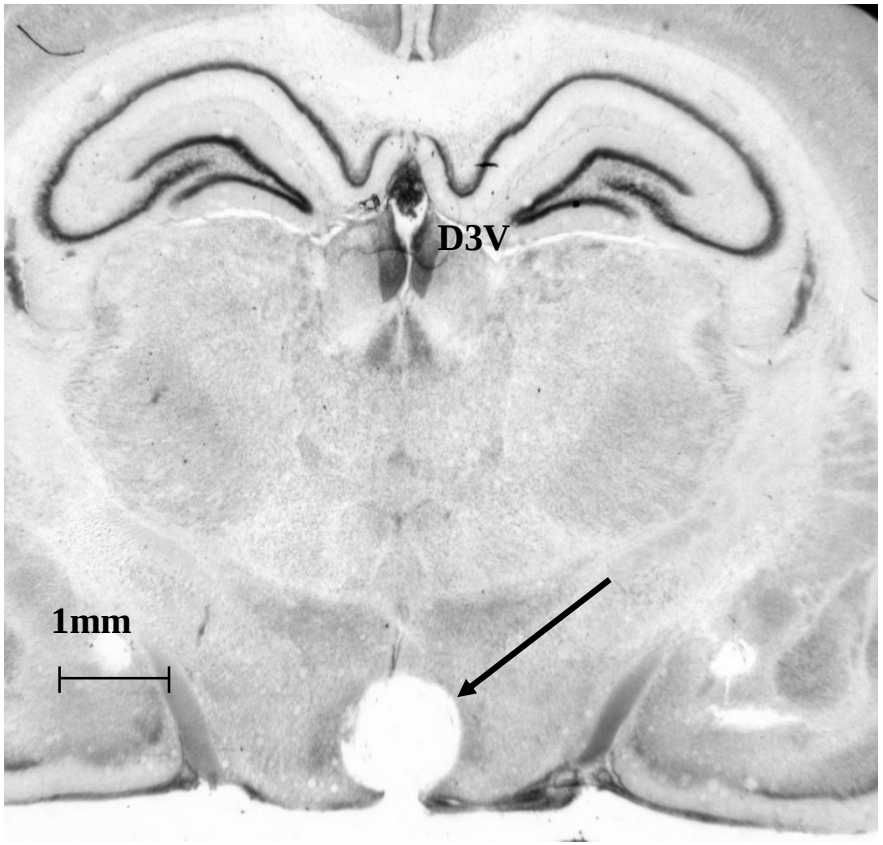
Dungan, H. M. et al. Endocrinology 2006

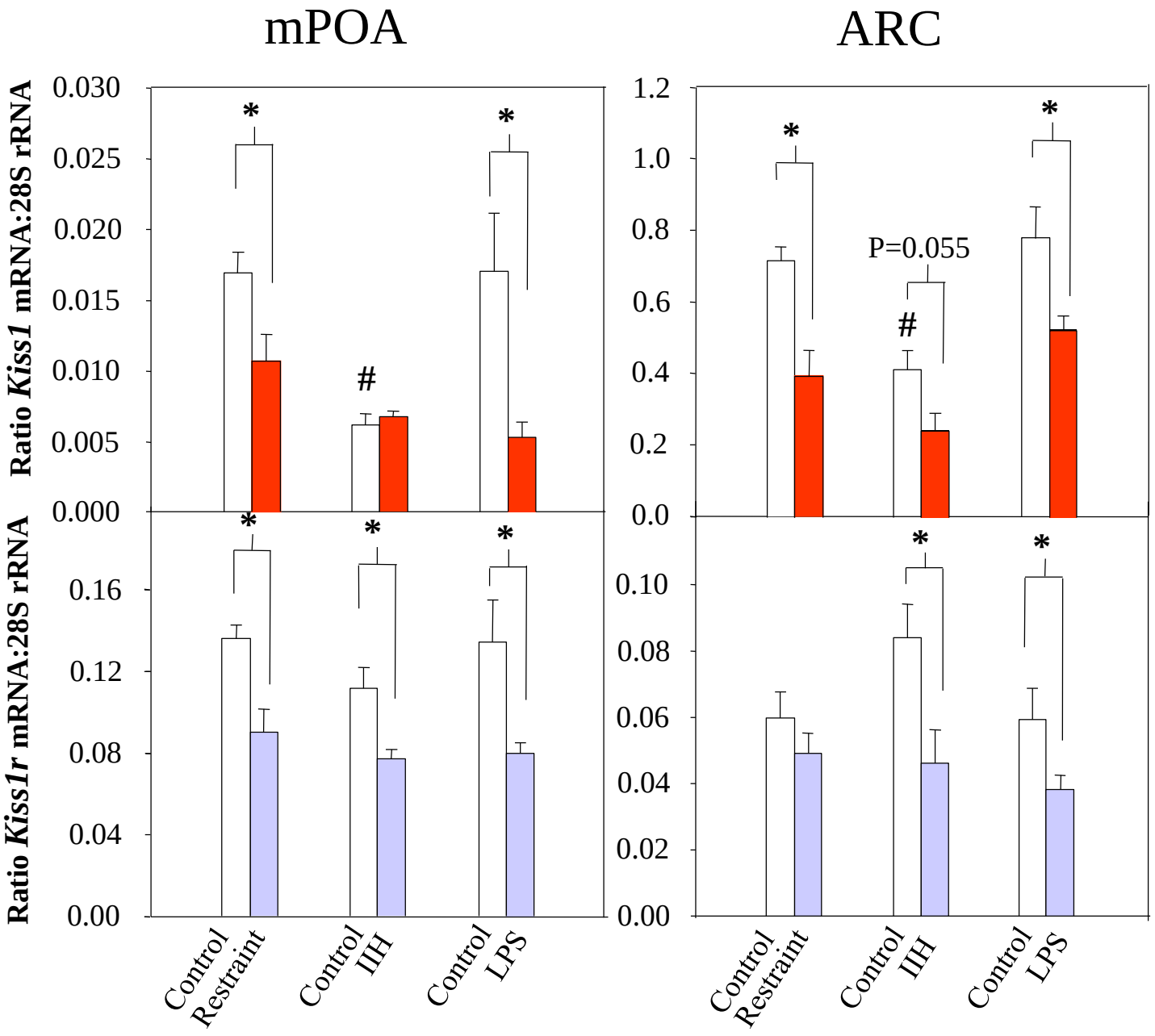
Micropunch method of Palkovits *versus* whole hypothalamus

mPOA



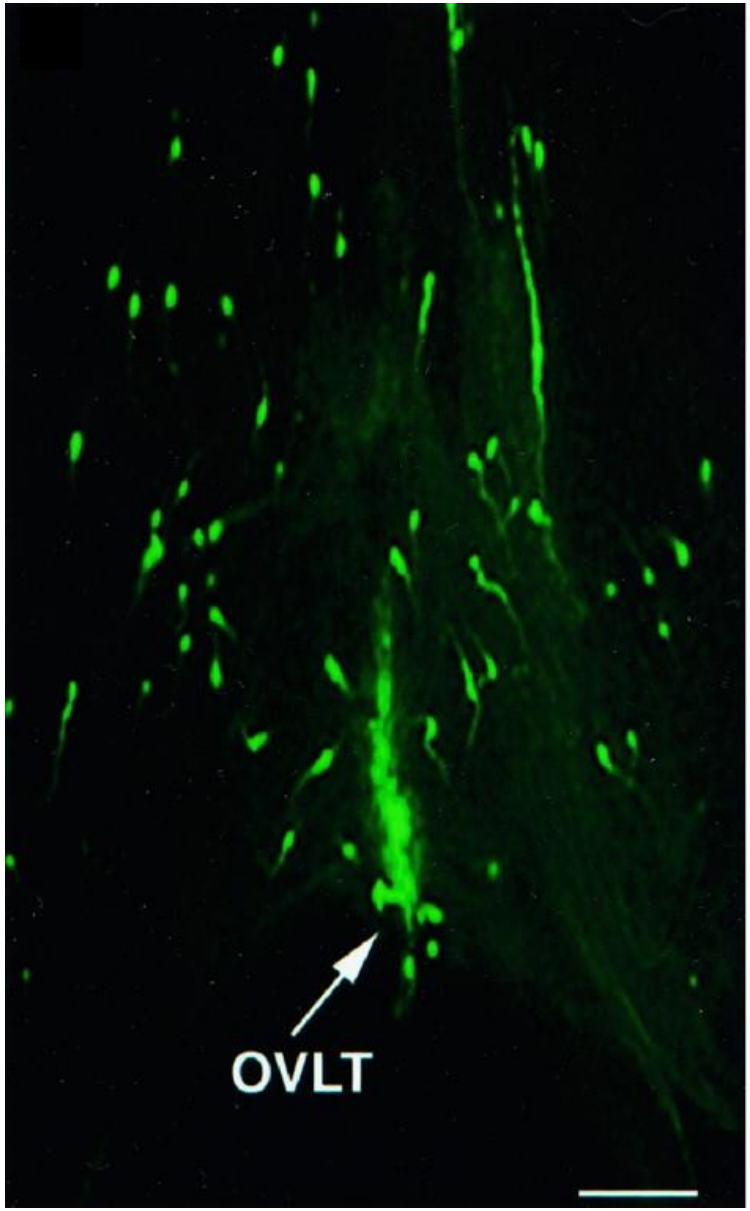
ARC





Kinsey-Jones. J. et al. J Neuroendocrinology 2008

GFP labelled GnRH neurones

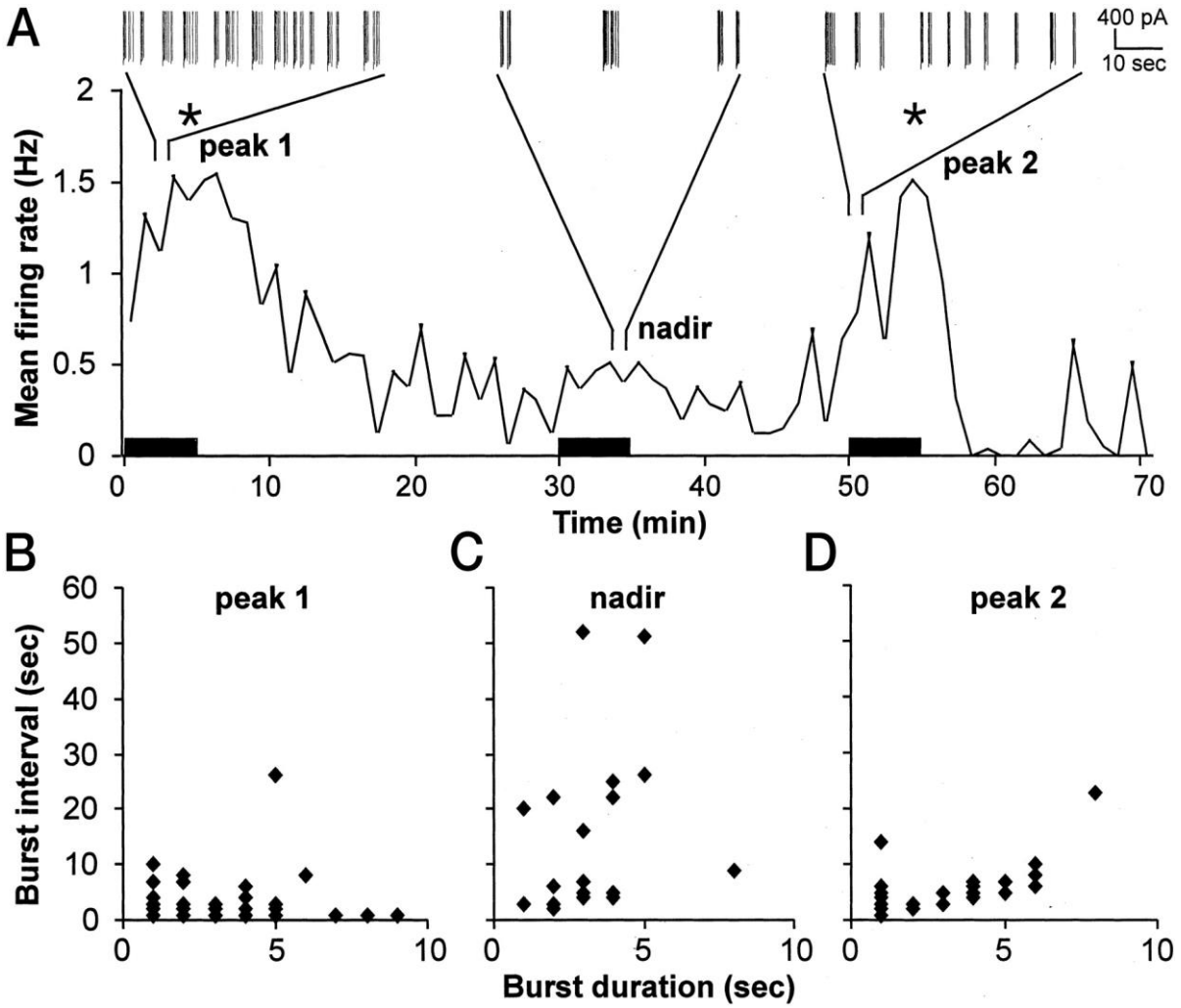


Suter, K. J. et al. Endocrinology 2000

Suter, K. J. et al. Endocrinology 2000;141:3731-3736

Endocrinology

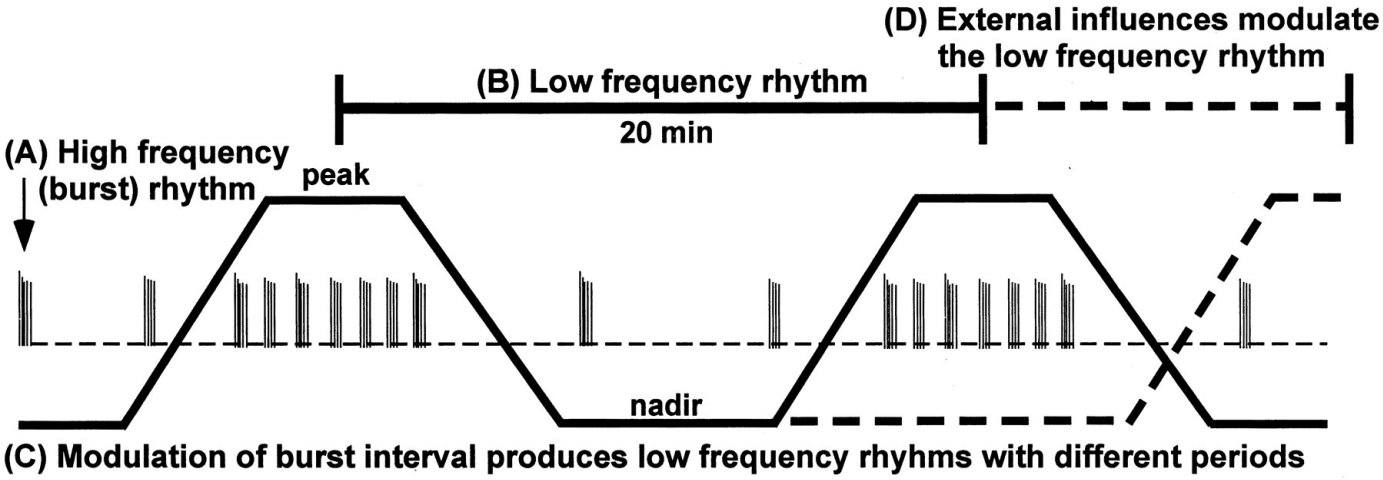
Burst interval is altered to produce peaks and nadir in firing rate



Nunemaker, C. S. et al. Endocrinology 2003;144:823-831

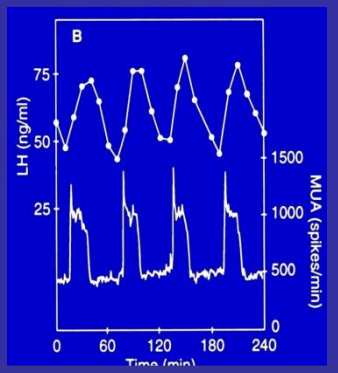
Endocrinology

Model of interacting rhythms in GnRH neurones (GnRH pulse frequency)



Nunemaker, C. S. et al. Endocrinology 2003;144:823-831

MUA recordings in the rhesus monkey



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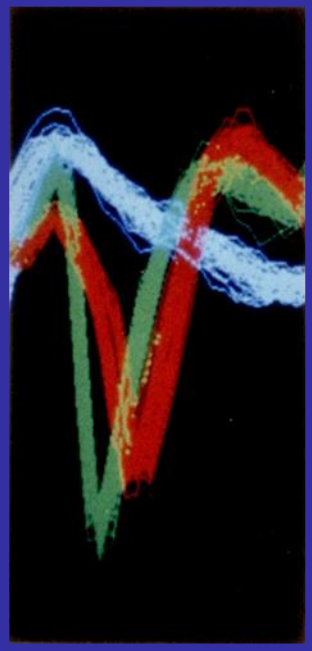
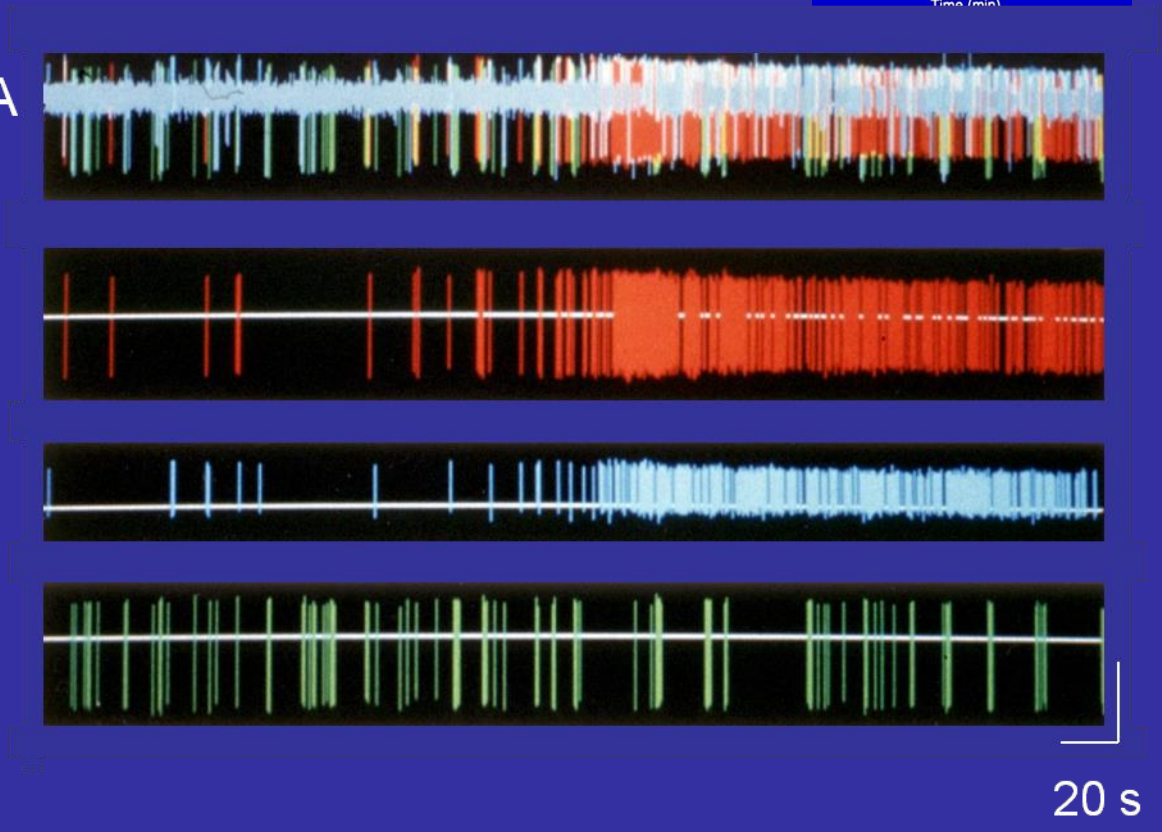
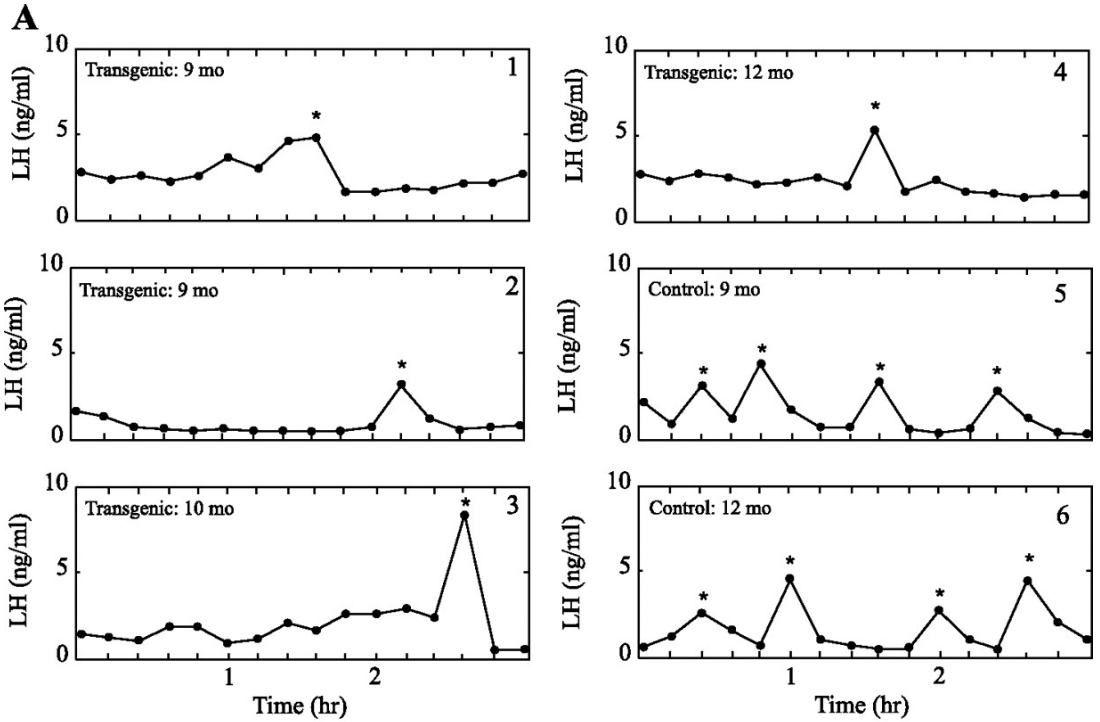
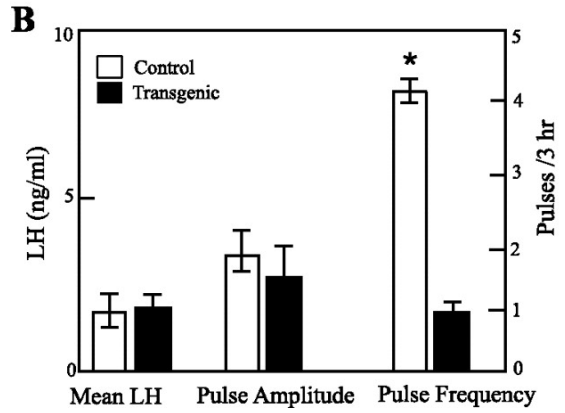


B



LH secretion in ovariectomized female mice of GnRH-GFP and non-transgenic controls





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