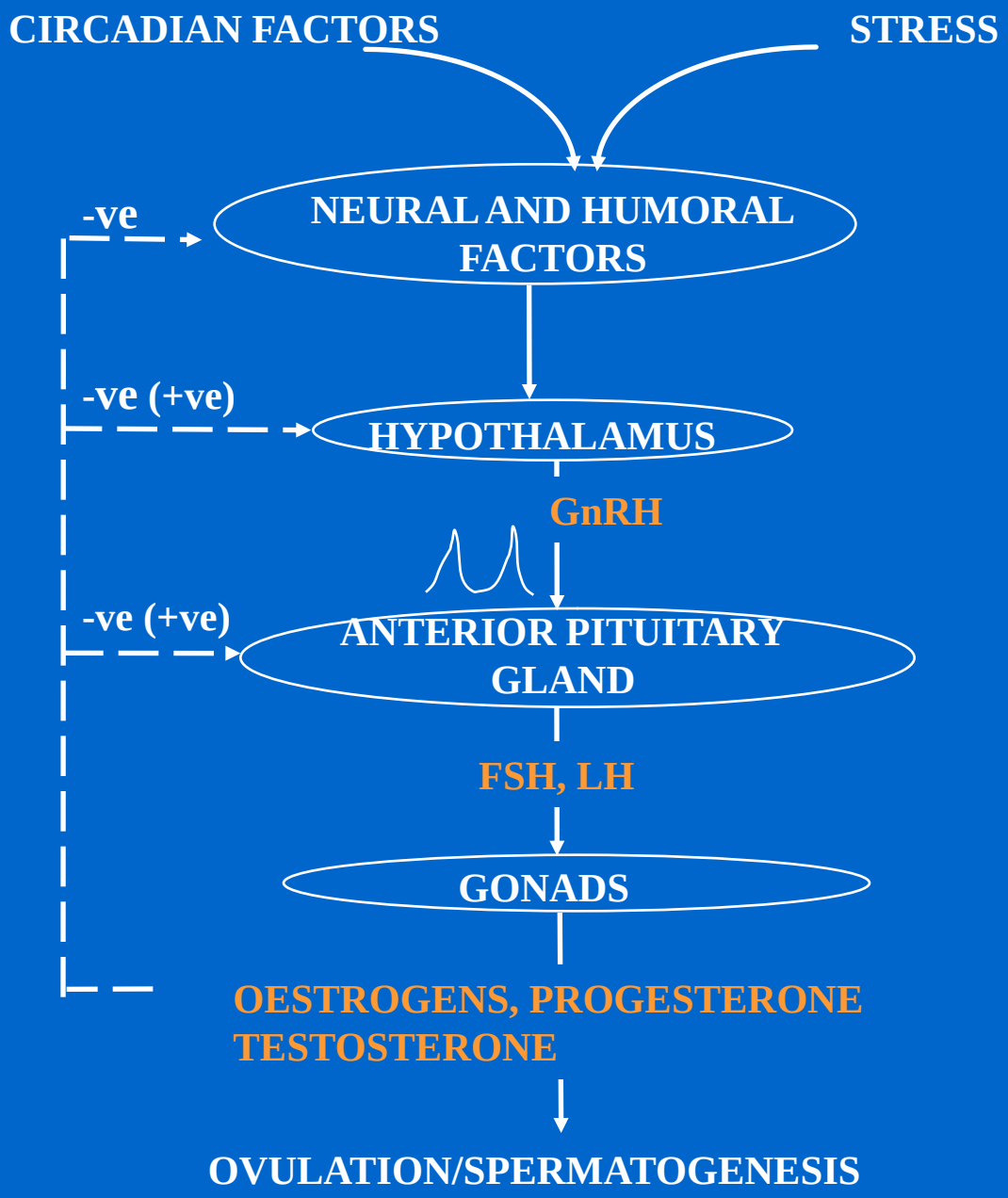
HPG AXIS , STRESS HORMONES AND REPRODUCTIVE FUNCTION

Dr P Cover

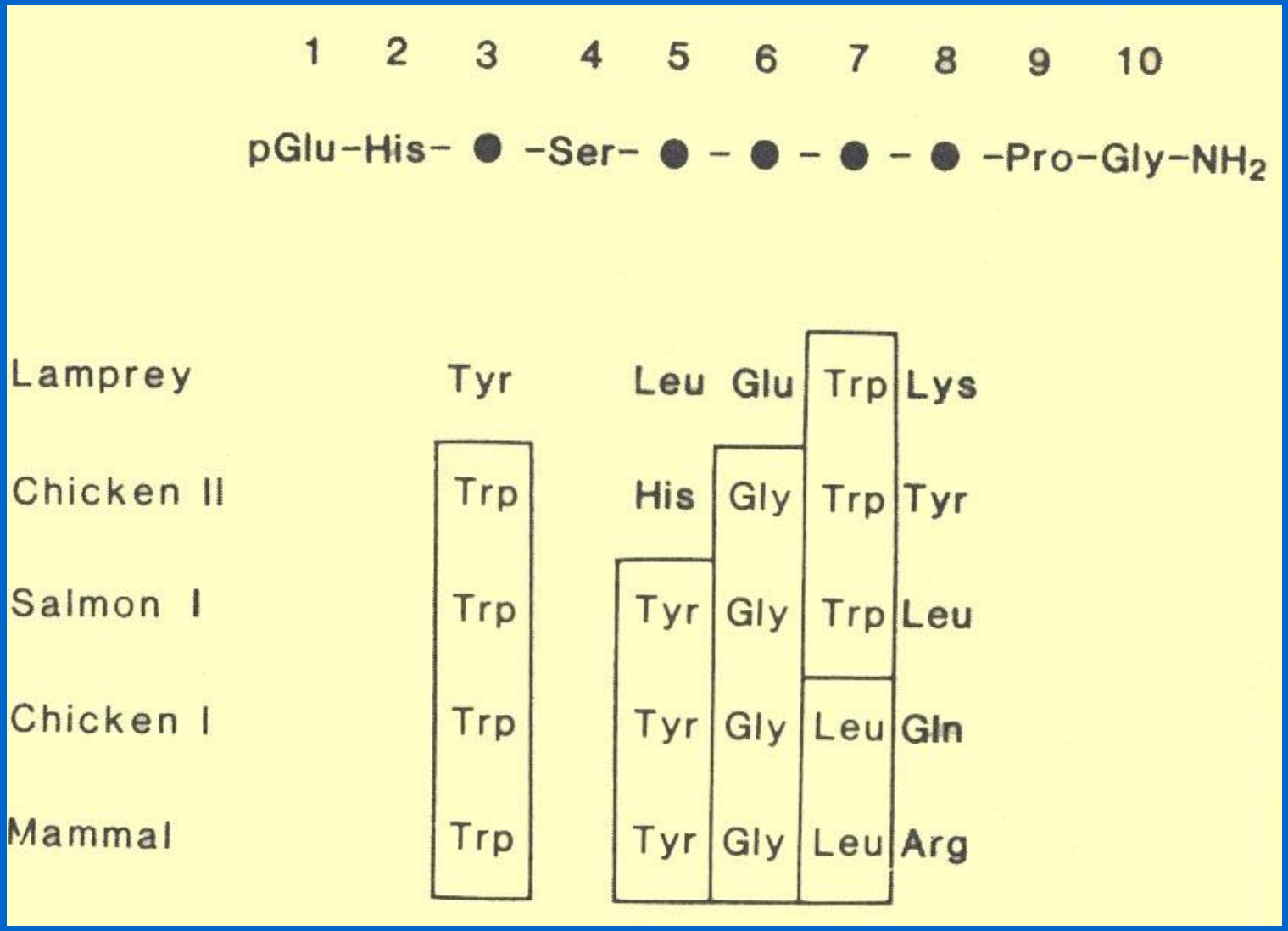
Learning objectives

1. Review the regulation of the HPG and HPA axes.
2. Discuss the regulation of GnRH secretion.
3. Describe some of the experimental models used to study the axes.
4. Describe the effects of CRH, AVP and glucocorticoids on the activity of the HPG axis.
5. Discuss their sites of action.
6. Discuss possible mechanisms/mediators of these effects.

Control of the Reproductive axis

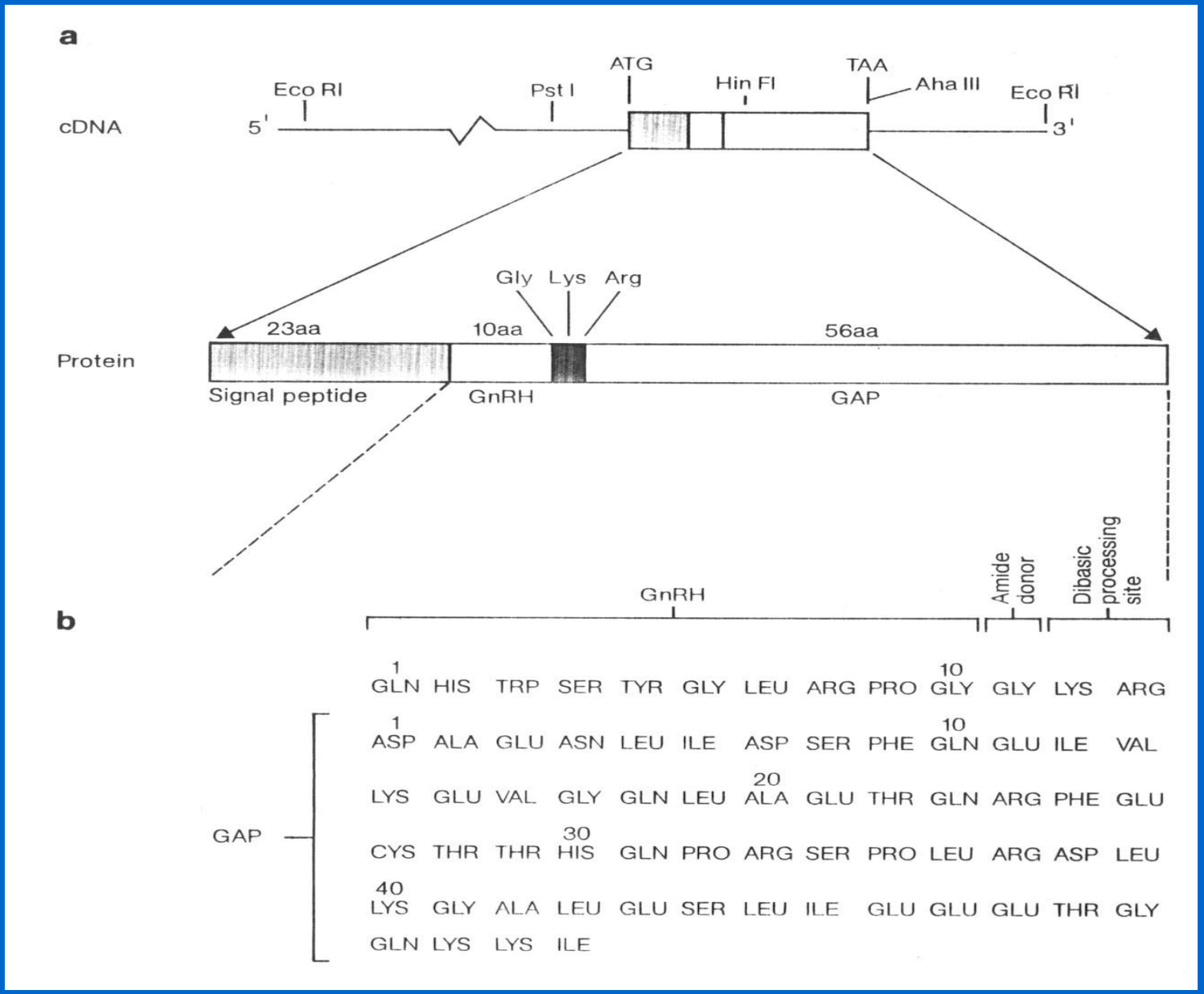


Naturally occurring GnRHs



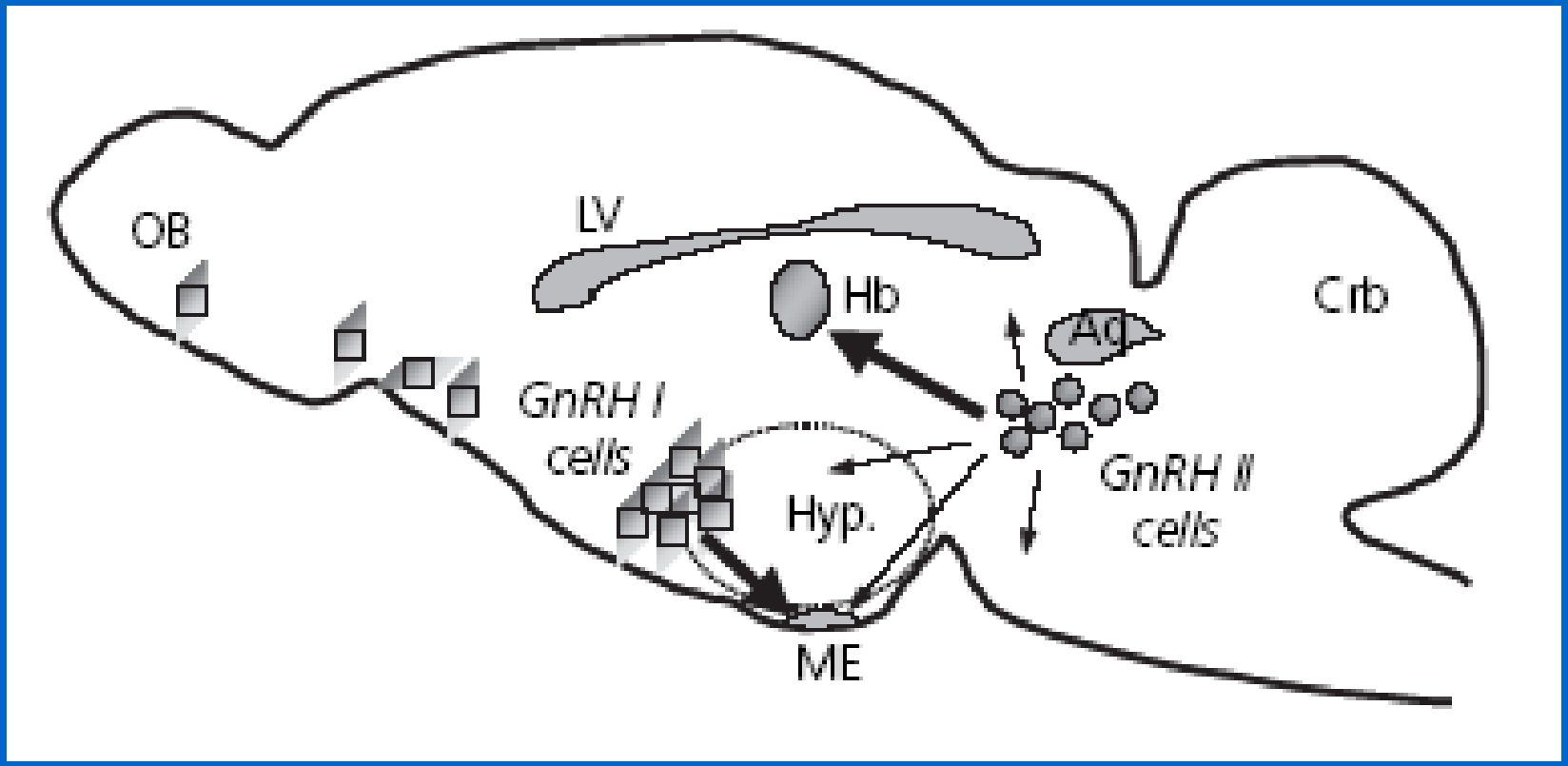
GnRH II

GnRH I



Sherwood et al 1994

Locations of (GnRH) I (□) and GnRH II (o) neurones.



Kauffman 2004

Major actions of GnRH

1. Stimulation of gonadotrophin release

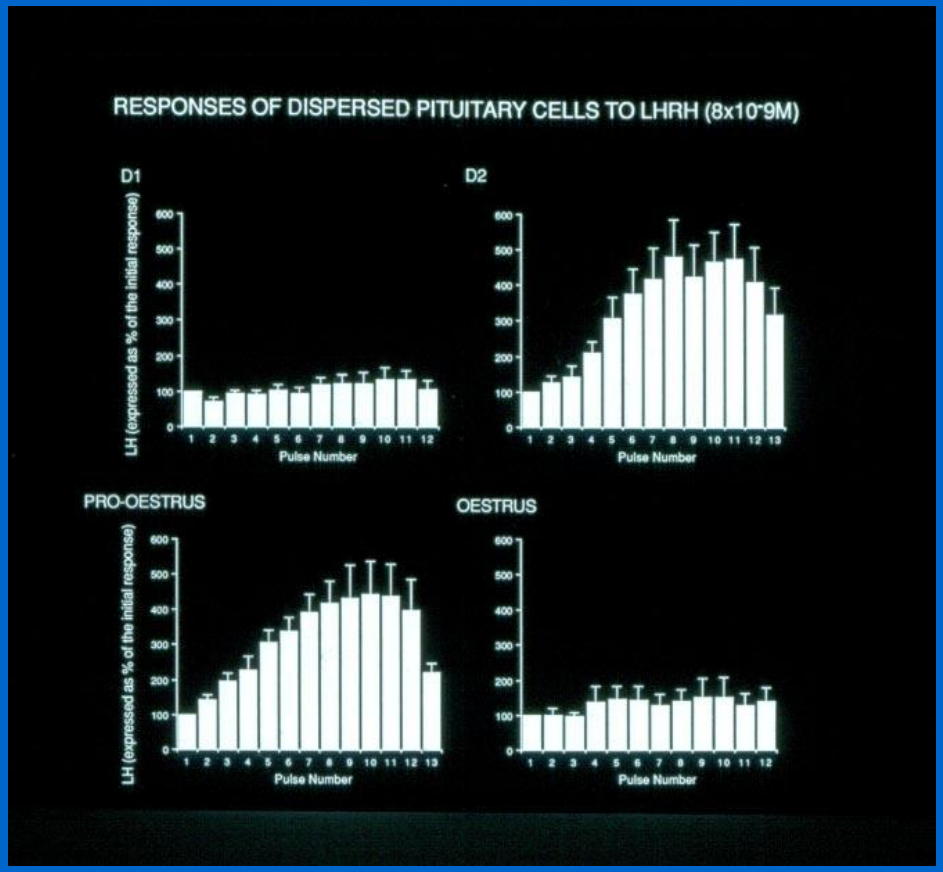
2. To prime the gonadotroph

3. Stimulation of gonadotrophin synthesis

4. Induction and maintenance of the secretory morphology of the gonadotrophs

2. Priming - ability to increase the responsiveness of the gonadotroph to itself.

Dependent on oestrogen environment



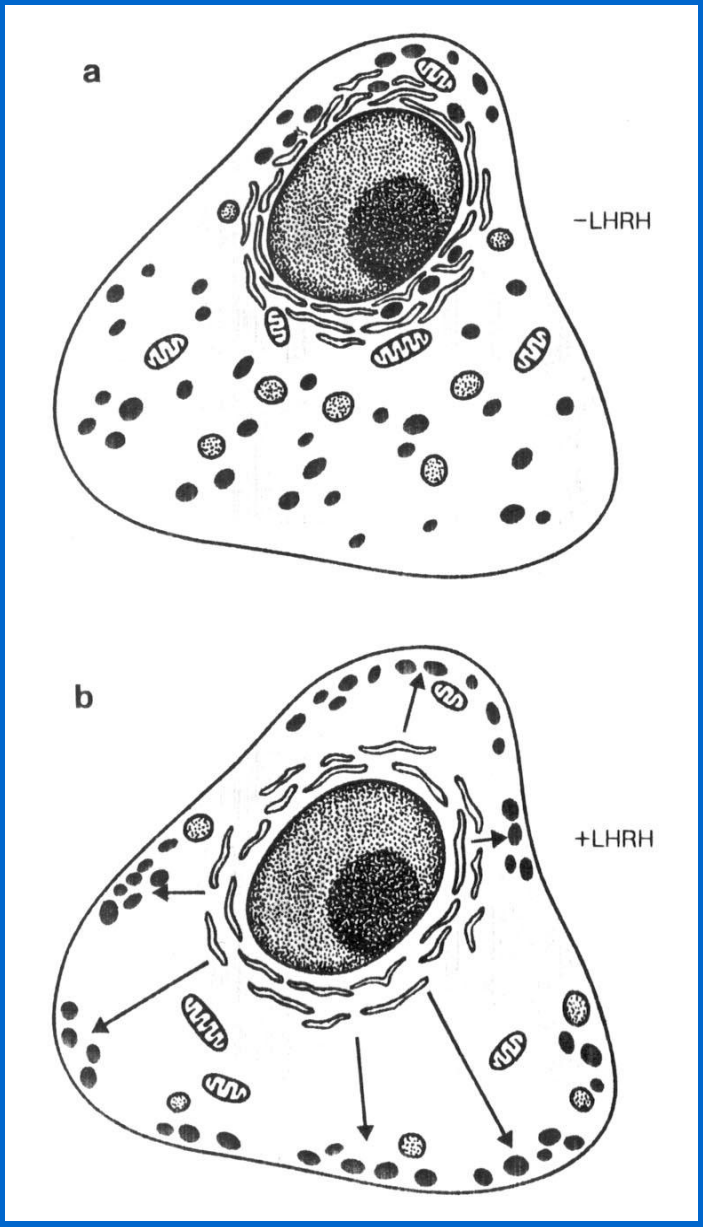
Mechanism :

1. Increase in no. of pit receptors

2. Alteration in activity of second messenger system.

3. Increase in the size of readily releaseable pool LH

4. Increase protein synthesis



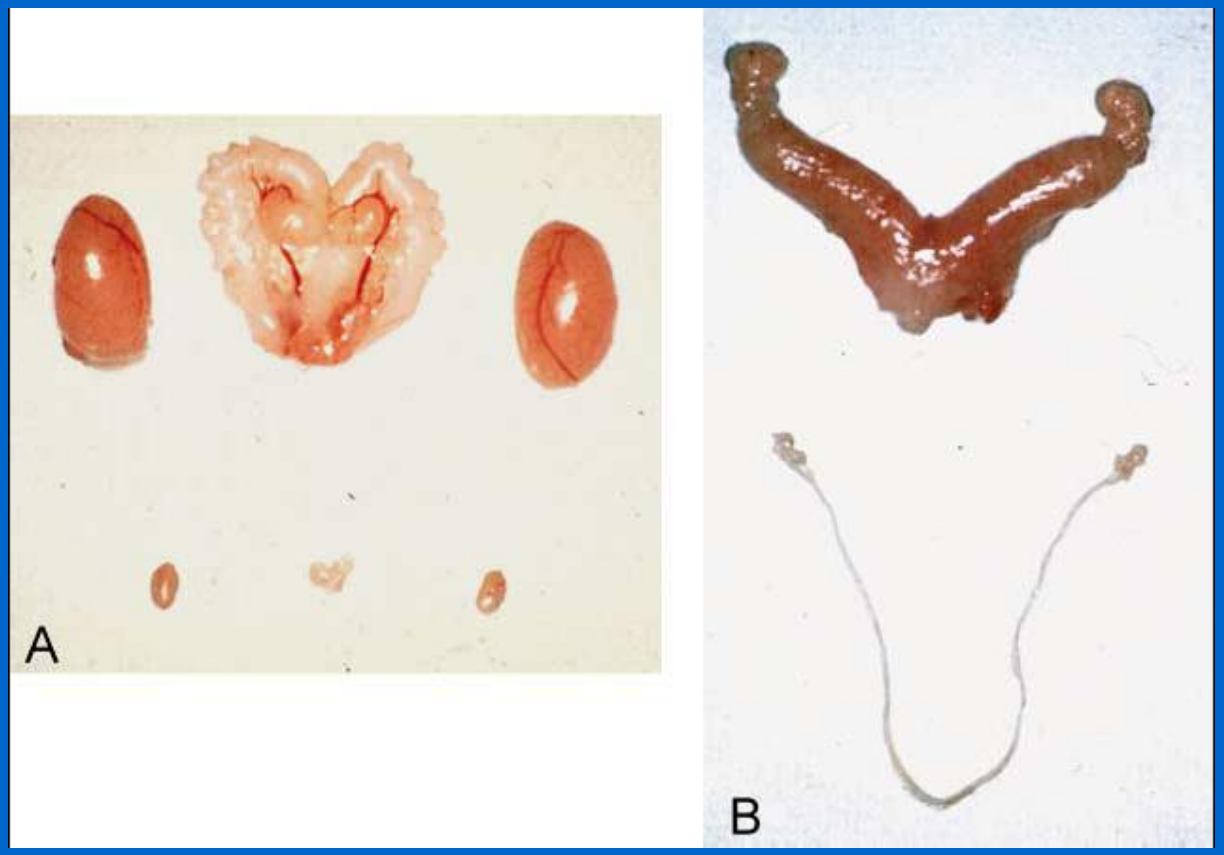
Gonadotrophin synthesis and maintenance of secretory morphology -

Trophic actions of GnRH:-

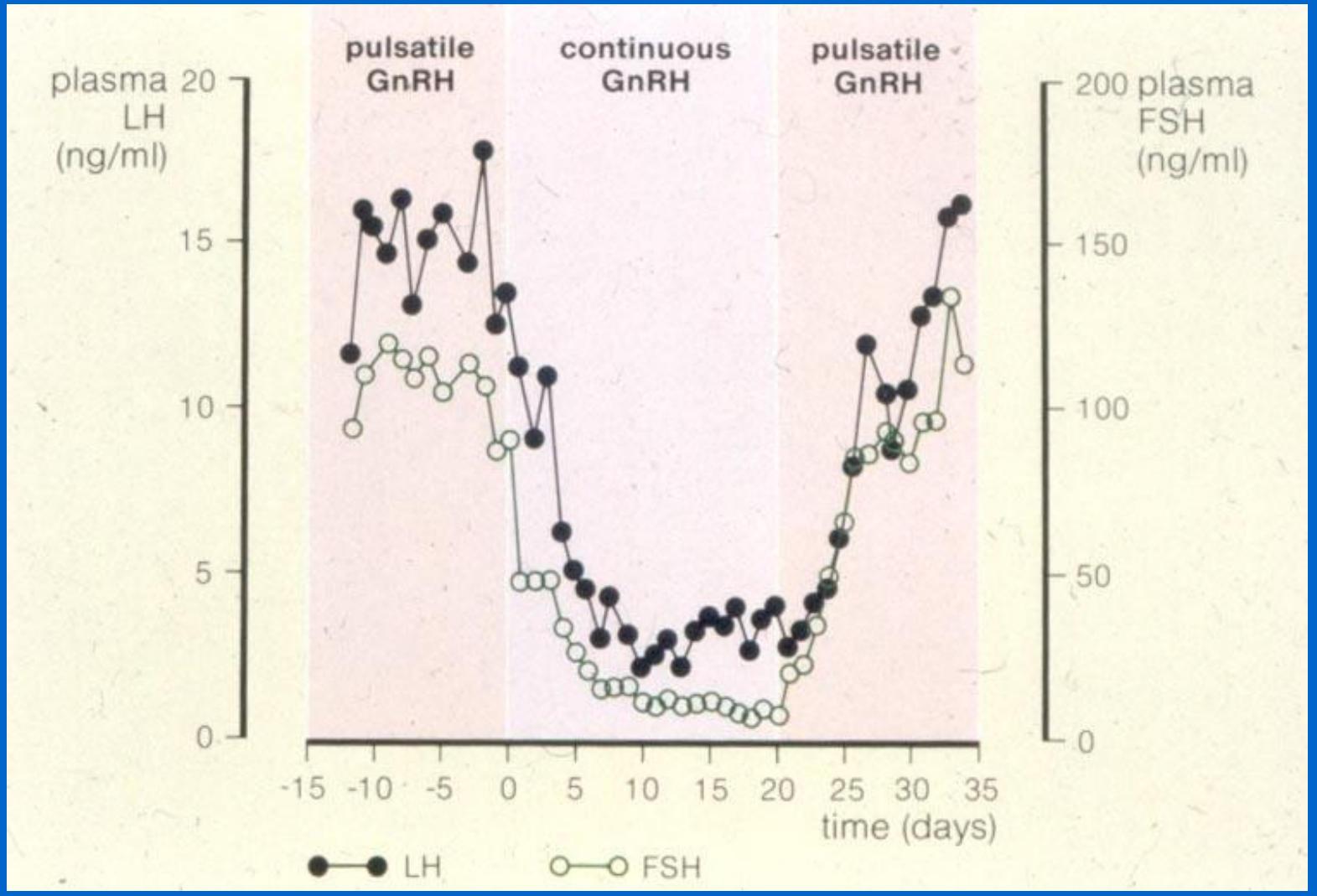
- ↑ GnRH receptors
- ↑number and size of gonadotroph
- ↑mRNA & protein
- ↑glycosylation

  Demonstrated in the hypogonadal (hpg) mouse

The hypogonadal (hpg) mouse

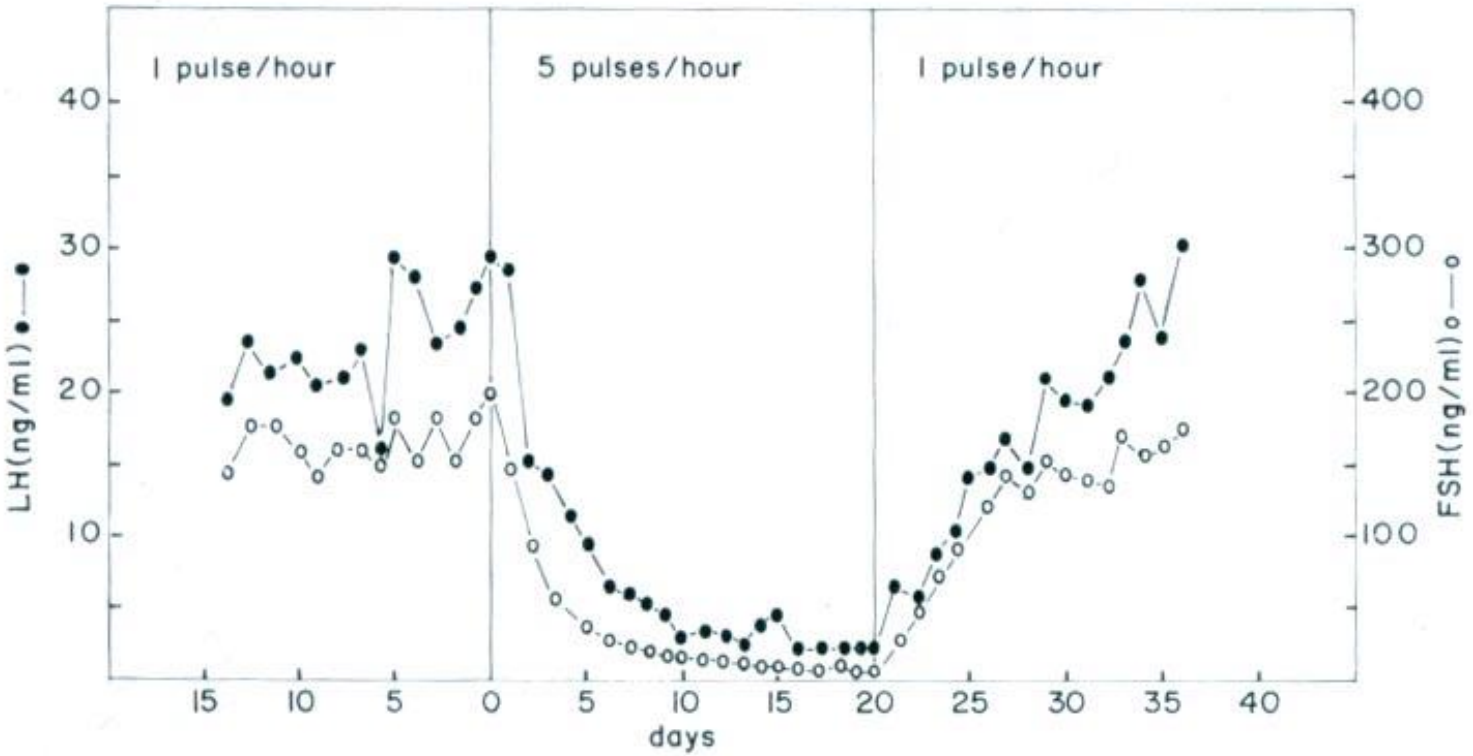


H Charlton, Reproduction (2004) 127 3–12



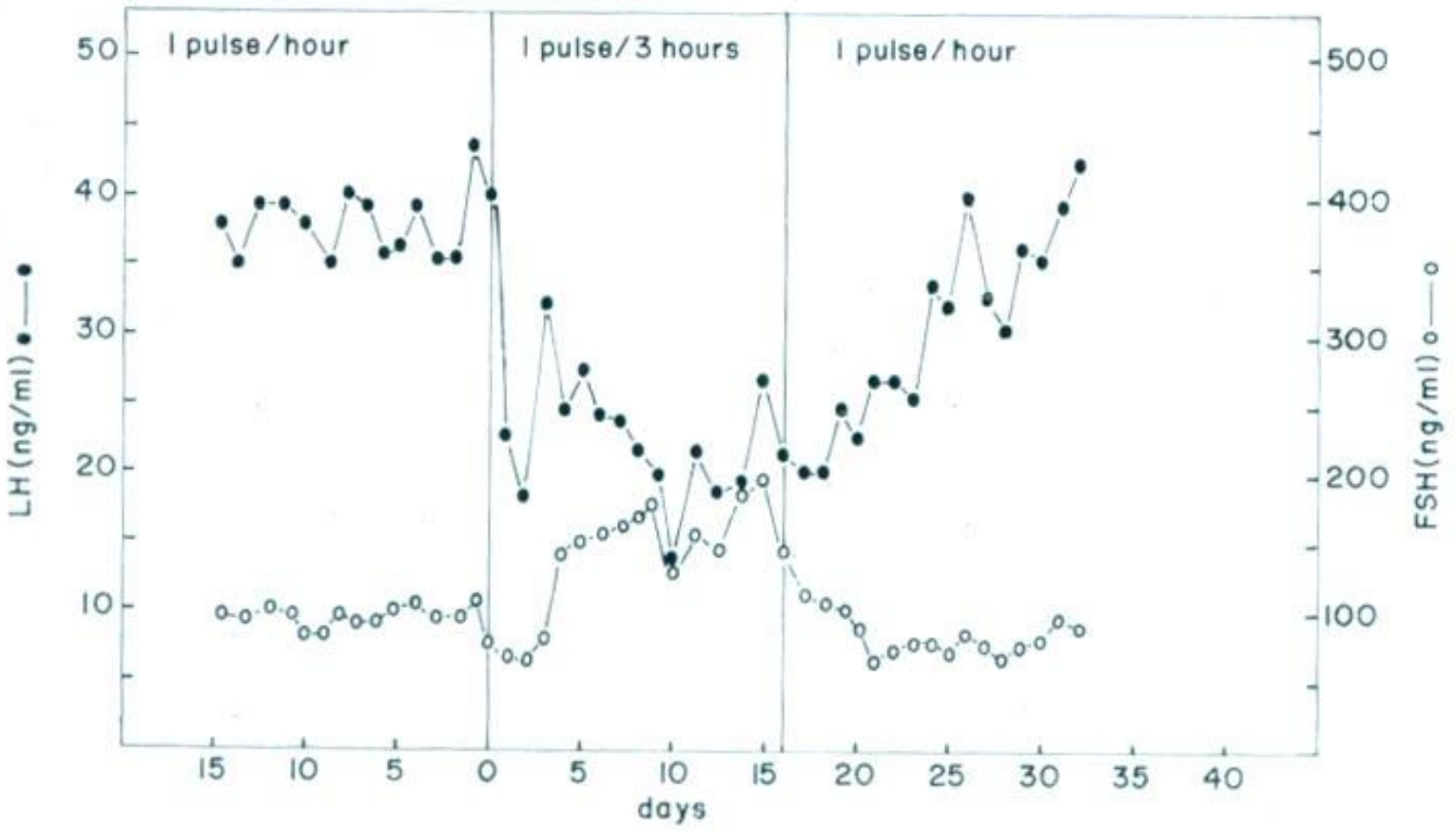
Knobil & colleagues

PLASMA CONCENTRATIONS OF LUTEINIZING HORMONE(LH) AND FOLLICLE STIMULATING HORMONE(FSH) IN HYPOTHALAMIC-LESIONED RHESUS MONKEYS RECEIVING PULSATILE INFUSIONS OF LUTEINIZING HORMONE RELEASING HORMONE(LHRH, 1 μg/min for 6 min)



Knobil & colleagues

PLASMA CONCENTRATIONS OF LUTEINIZING HORMONE(LH) AND FOLLICLE STIMULATING HORMONE(FSH) IN HYPOTHALAMIC-LESIONED RHESUS MONKEYS RECEIVING PULSATILE INFUSIONS OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH, 1 μg/min for 6 min)



Knobil & colleagues

- Pulsatile pattern is ESSENTIAL for normal functional activity of the gonadotroph.
- Over exposure of the gonadotrophs to GnRH renders the cells refractory to stimulation.
- Under certain conditions intermittent exposure can enhance Gonadotrophin release in response to subsequent stimulation i.e. sensitization or ‘priming’ effect.

Kisspeptin

- Group of peptides (Kisspeptin 54 – metastin)
- Binds to GPR54 – mutation – hypogonadotropic hypogonadism
- Induces Gonadotrophin secretion
- Kisspeptin neurons in ARC & lateral hypo (POA /AVPV)
- GnRH neurons express KP receptors

The GnRH pulse generator

KNDy neurons - 3 peptides shown to be co-localised in a single subpopulation in the ARC (sheep)

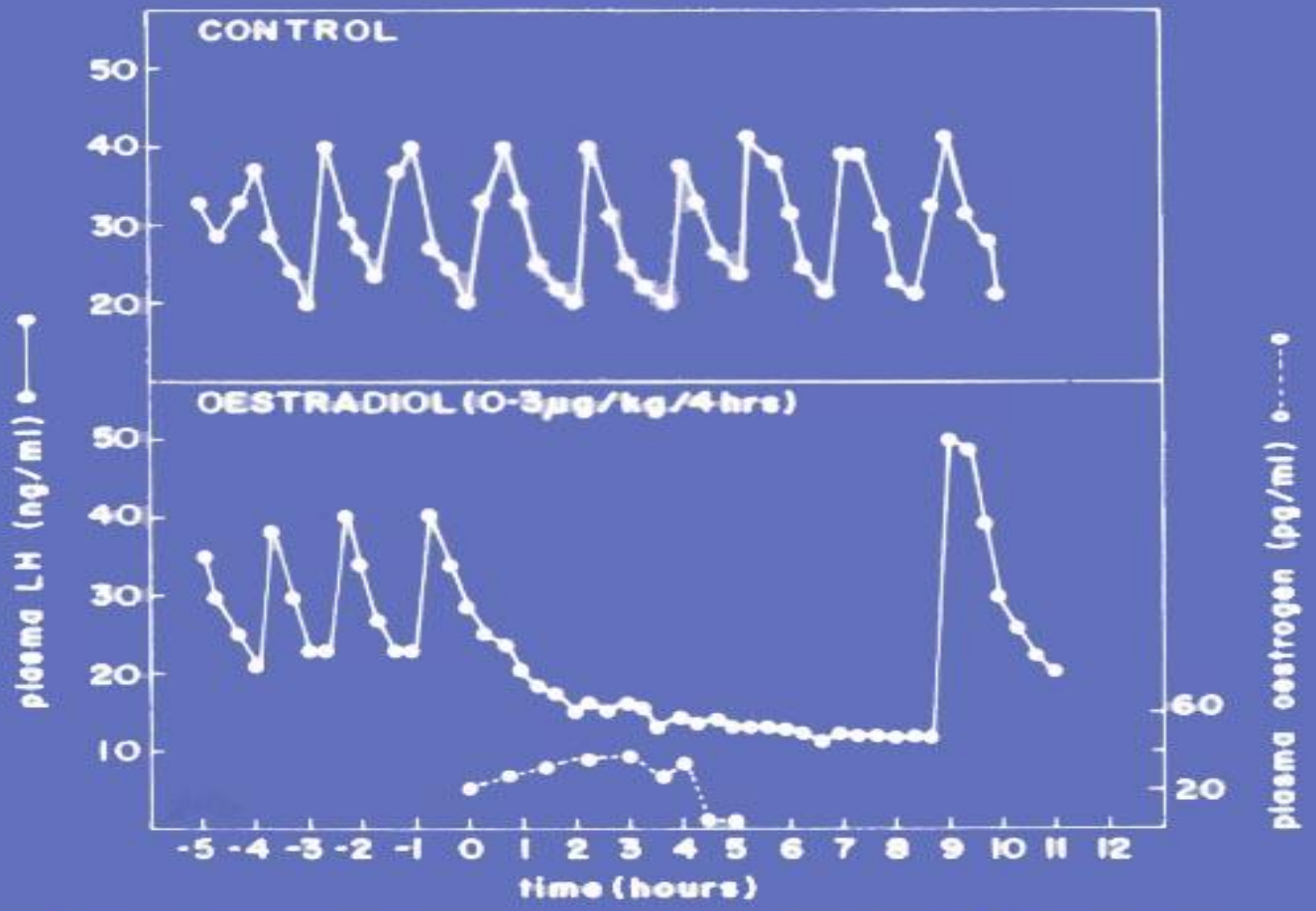
K - Kisspeptin

N - Neurokinin B (NKB)

Dy - Dynorphin (DYN)

K + NKB essential for normal GnRH secretion in humans
(Mutations in peptides or receptors →hypogonadism)

INHIBITION OF EPISODIC LH SECRETION IN OVARIECTOMIZED RHESUS MONKIES BY INFUSION OF LOW, PHYSIOLOGICAL DOSES OF OESTRADIOL.



Plasma oestrogen concentrations prior to infusion were undectable.

FROM: Yamaji, T. et al (1972) Endocrinology 90, 771-777.

Negative feedback

Hypothalamus

- Steroid implants into MBH suppress LH & FSH, reverse the increase in pro-GnRH mRNA induced by ovariectomy, alter GnRH neuronal activity.

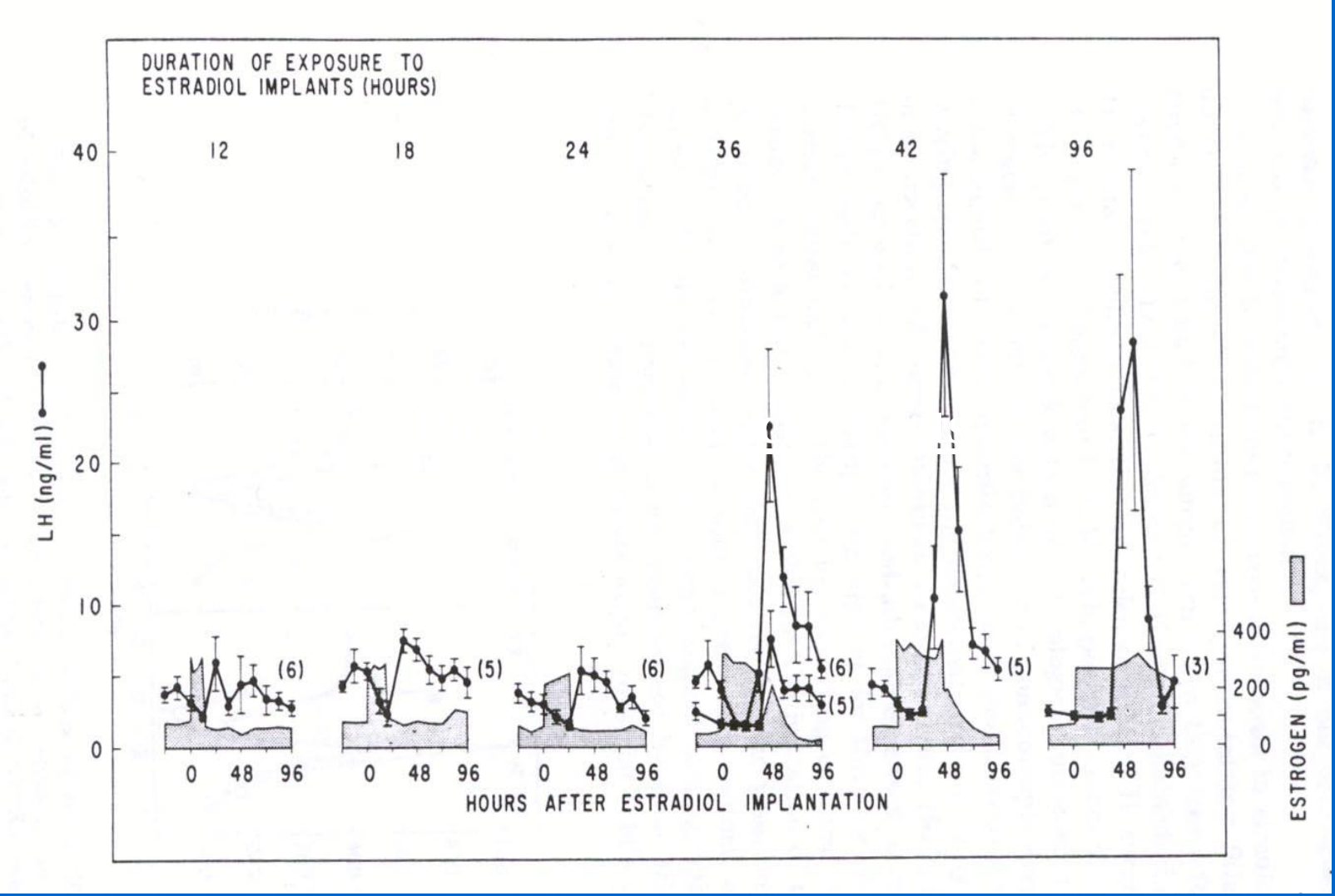
(but effects probably not exerted directly on GnRH neuron but by other neuronal systems (e.g. GABA, DA, AD also NPY, B-end) which have E2 receptors.

- Kisspeptin neurons in ARC mediate the –ve feedback actions of E2 (express ER).

Pituitary

- negative feedback effects in lesioned animals
- inhibits secretion of LH by pituitary tissue *in vitro*

Positive feedback effects



Karsch et al 1973

Where is the positive feedback effect exerted?

Hypothalamus

- implantation of E2 in POA results in LH release
- GnRH pulse frequency in increased at pro-oestrus.
- mainly indirect via other neuronal systems with sex steroids receptors?

- In rodents - AVPV K neurons important
- In sheep and primates MBH,lateral hypothal and POA

Positive feedback pituitary

- In late follicular phase increased concentrations of E2 associated with increased pituitary sensitivity
- exogenous E2 produce similar changes in responsiveness of gonadotrophs
- E2 can produce a LH surge in hypothalamic lesioned animals receiving regular pulses of GnRH
- Effects of E2 can be demonstrated using a pituitary column preparation

Pulses during the cycle

Follicular Phase

Early - low frequency (60-90min) moderate amplitude with suspension or slowing of pulse during sleep.

Mid - increased frequency (60min) and reduced amplitude. Sleep related alterations disappear.

Late - amplitude increases markedly.

Pulses during the cycle

Luteal phase

Early - slowing evident as progesterone rises (2-4h)

Mid - slow (4-6h)

End - LH pulse continues to slow until 1 or 2 large pulses in a 24h period, leads to luteolysis, termination of -ve feedback → change from low amplitude pulses to a more regular, higher amplitude pattern.

Progesterone slows the pulse frequency

Oestrogen reduces the pulse amplitude.

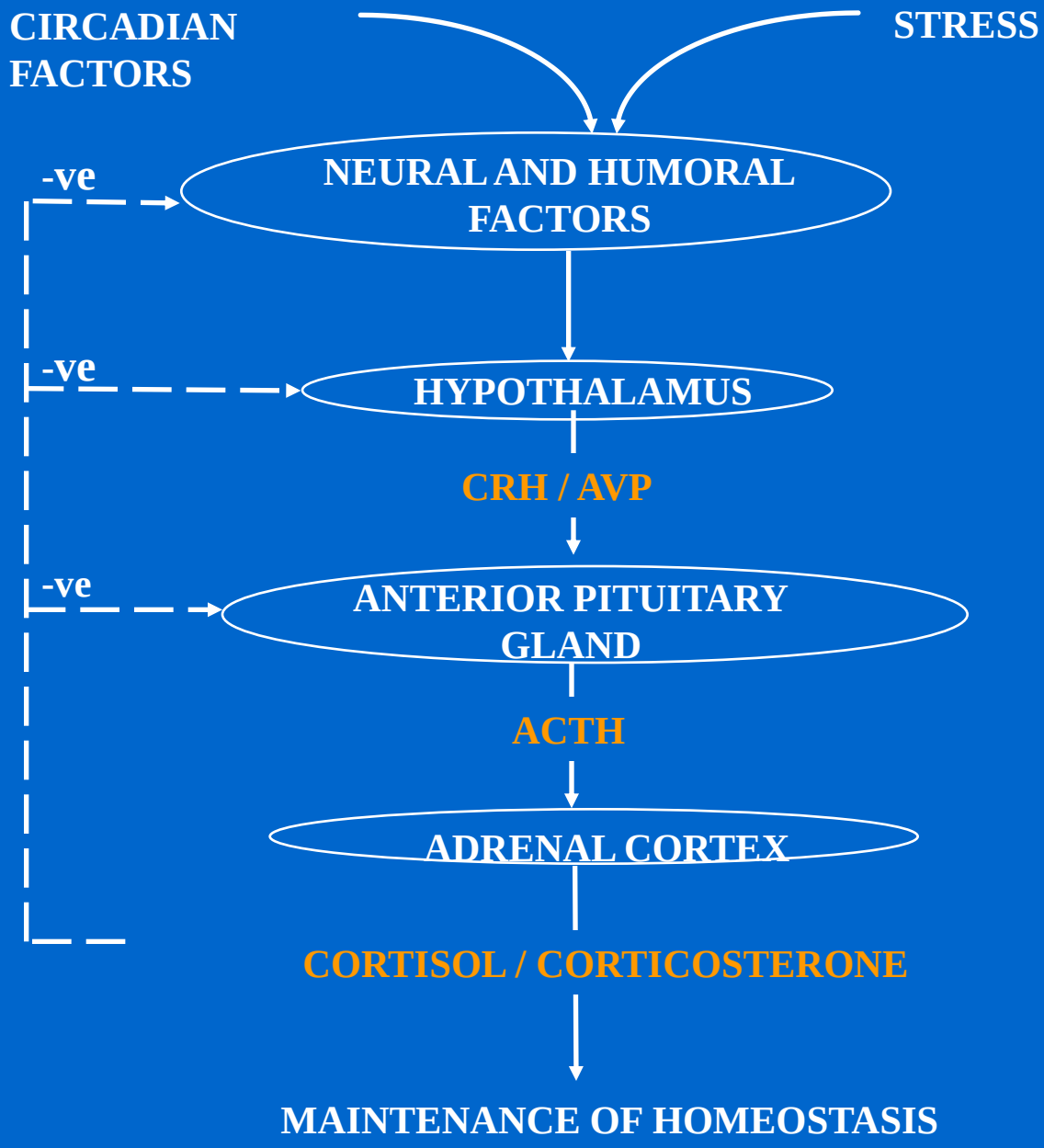
Stress can be defined as :-

- any threat, real or perceived that compromises homeostasis.

In the neuroendocrine context - stress is a stimulus that results in the activation of the HPA axis.

Stress can also disrupt reproductive function.

The hypothalamo-pituitary-adrenal axis



- Stress activates the HPA axis (stress response meant to be acute)

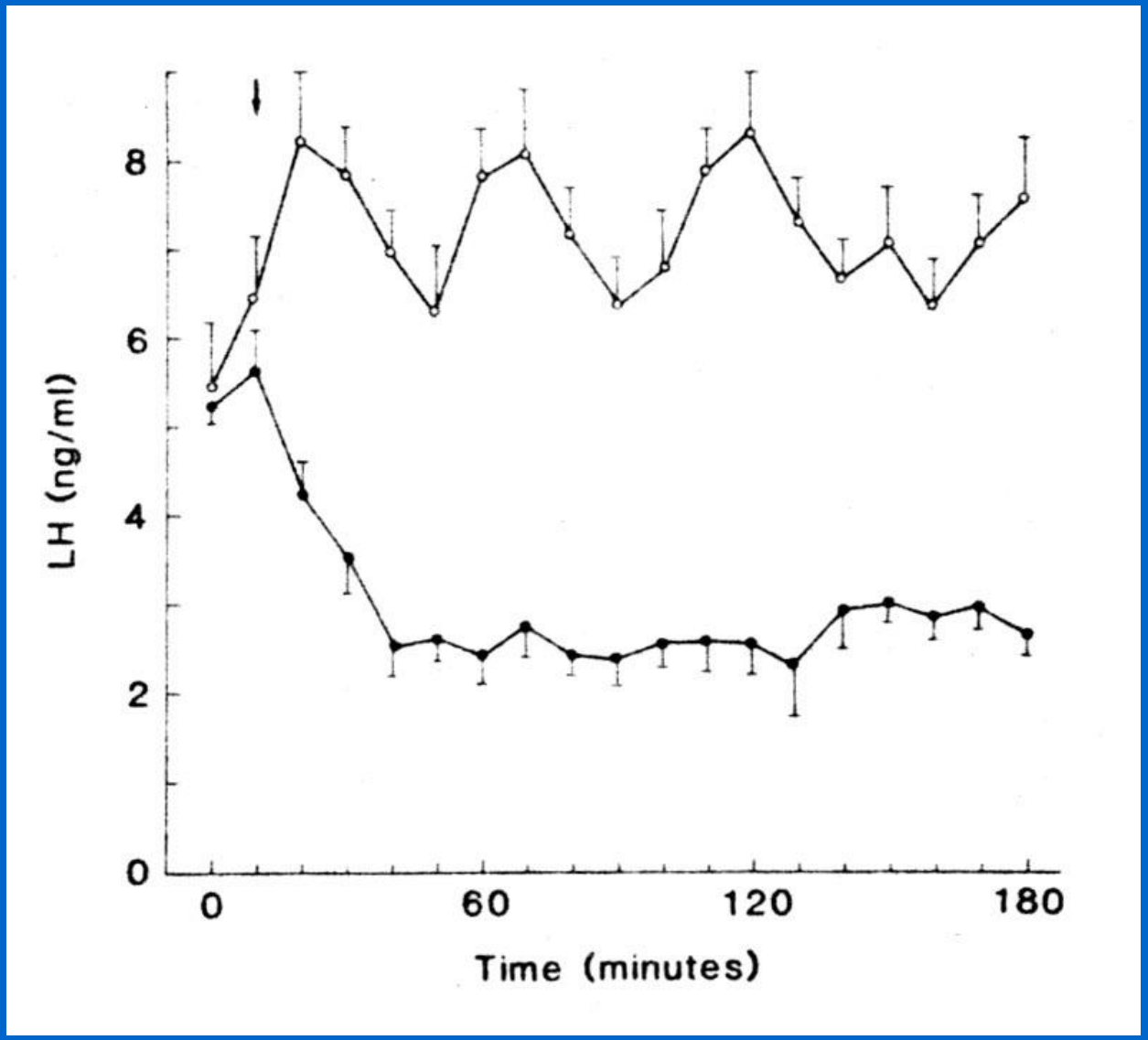
- Excessive activation of stress responses - stress related diseases (Selye 1936).

- Stress disrupts HPG axis - depression of gonadotrophin secretion (slowing of GnRH/LH pulse frequency), oligospermia, amenorrhoea and consequently infertility.

- Stress will disrupt other systems e.g. growth and immunity

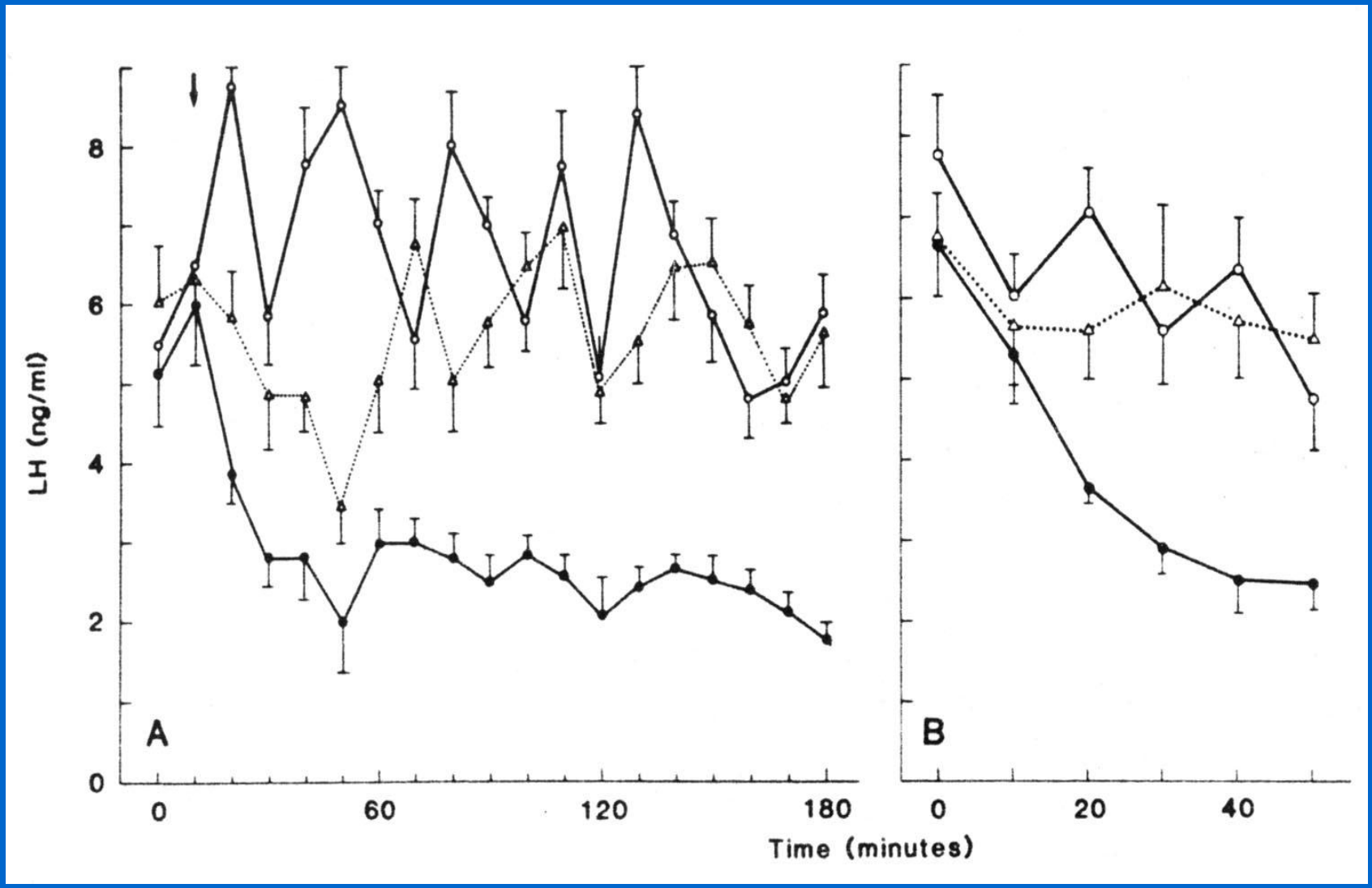
What is the nature of the stress?

Effect of stress on LH secretion

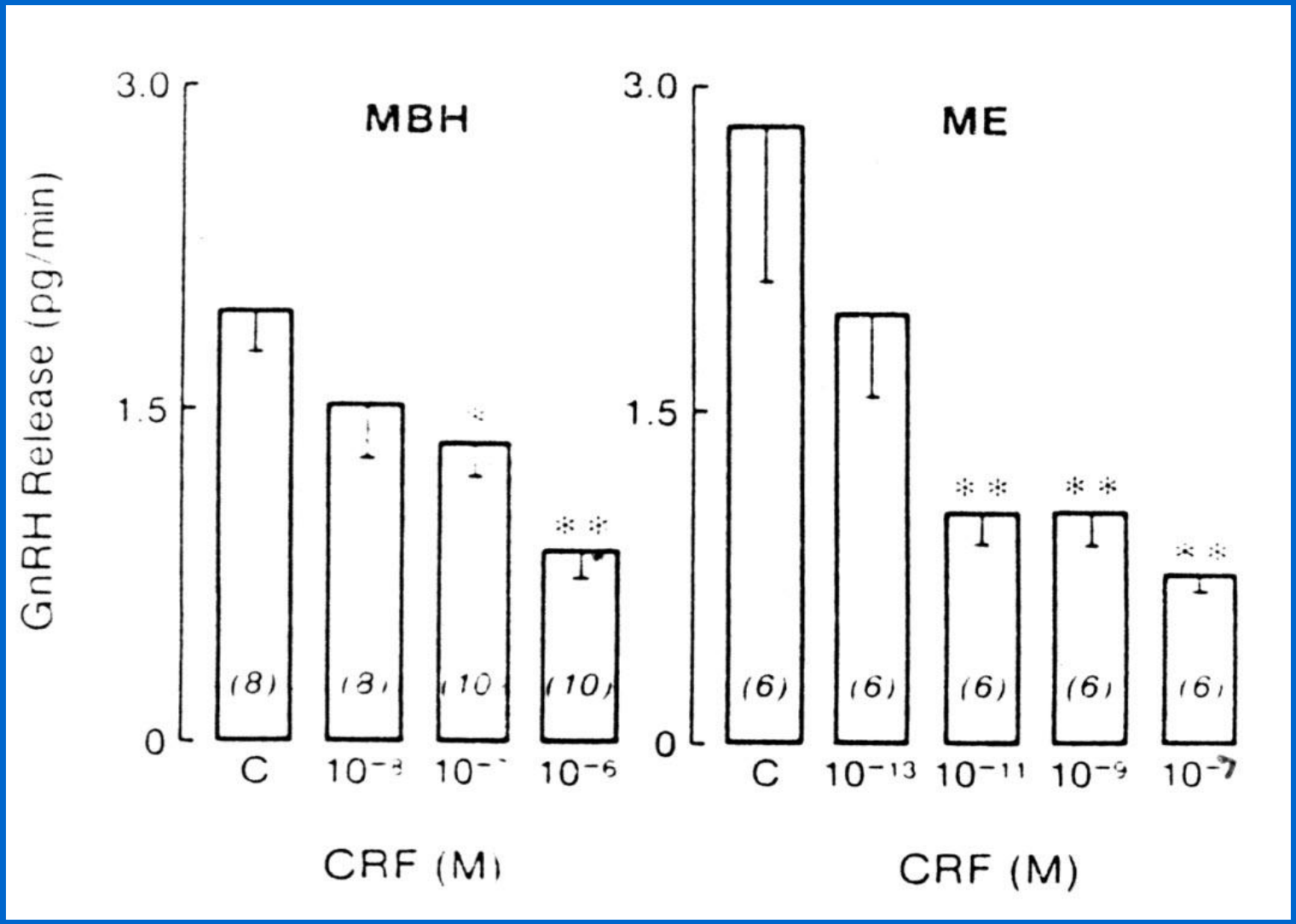


Rivier & Vale Science (1986) 231, 607-609

Reversal of stress effect -oCRF9-41

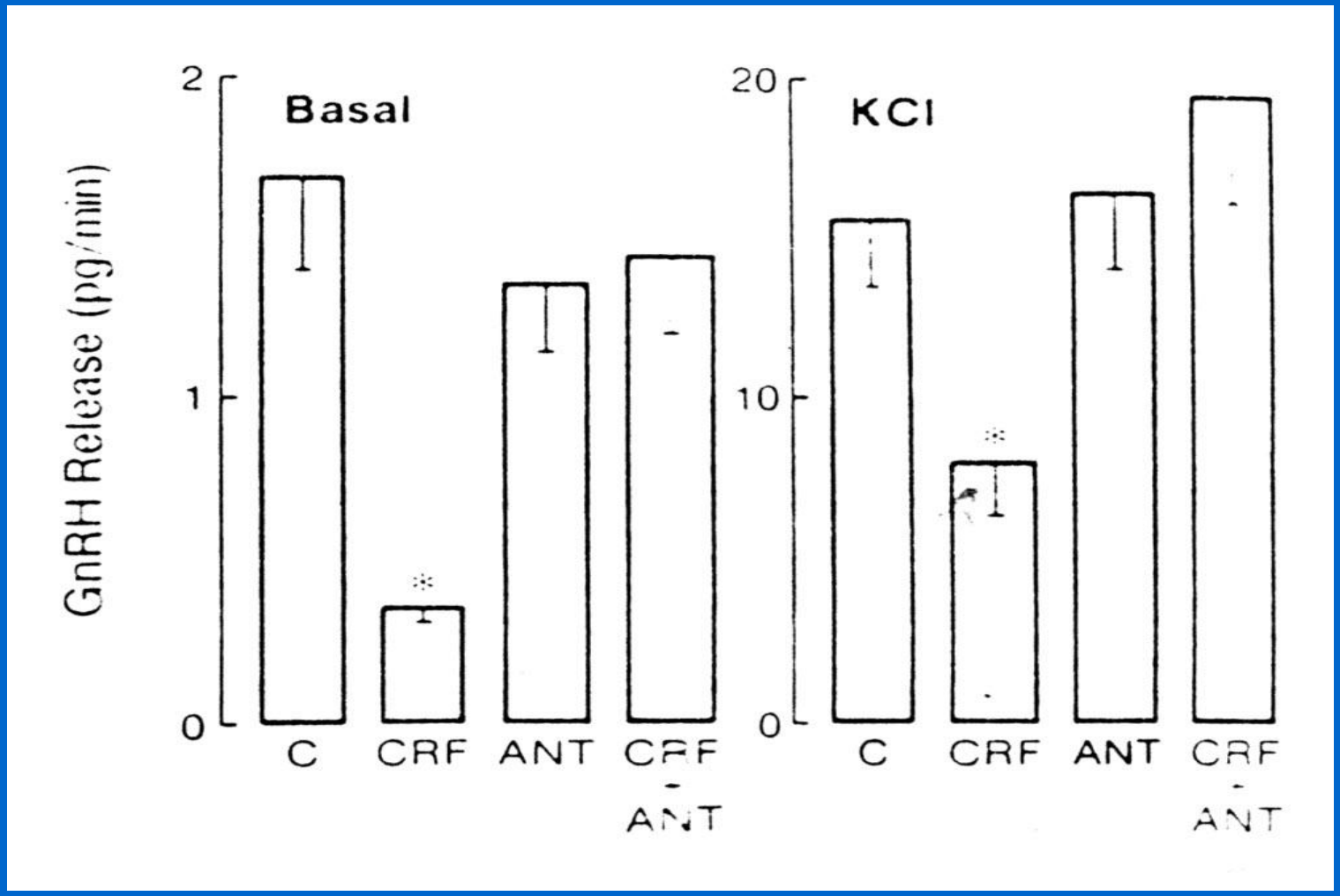


In vitro studies



Gambacciani et al

In vitro studies - antagonist



How does CRH mediate these effects?

1. Anatomical evidence - direct synaptic connections.

? Few CRH neurons project to POA from PVN.

Lesions in PVN fail to prevent stress induced inhibition of LH.

BNST (Bed nucleus of the Stria Terminalis)

Locus coeruleus

- Both contain high density of CRH neurons, implicated in GnRH secretion. Projections to POA (not CRH), probably act via interneurons.

2. Effects via other neuronal system

(a) Endogenous opioid peptides (EOP)

- Stress increases the activity of central opioidergic neurons
- Both EOP antagonist and CRH antagonist reverse the effects of stress
- CRH stimulates the release of β-endorphin and β-dynorphin
- Anti beta endorphin serum will abolish the effect of icv CRH
- Anatomical and functional associations CRH and EOP neurons

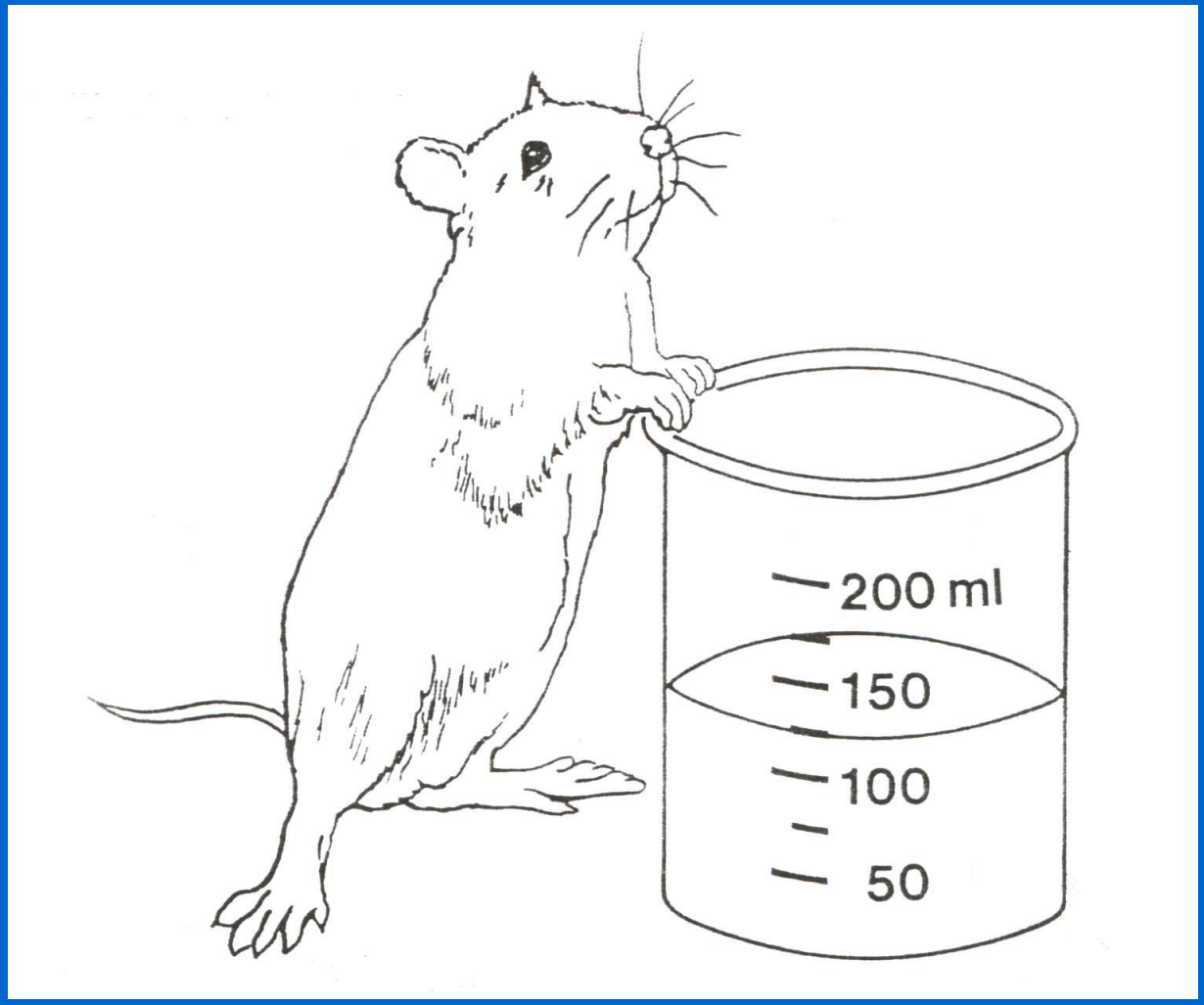
(b) Catecholamines

- Both opioid and CA-containing neurons project to areas rich in GnRH cell bodies.
- NA/A increase LH secretion in ovx+E2 rats
- reduced NA/A synthesis prevents both the pre-ovulatory LH surge and E_2 induced release of LH in female rats and block stress-induced suppression of LH
- Interactions between CRH and catecholamine-containing neurons (locus coeruleus)

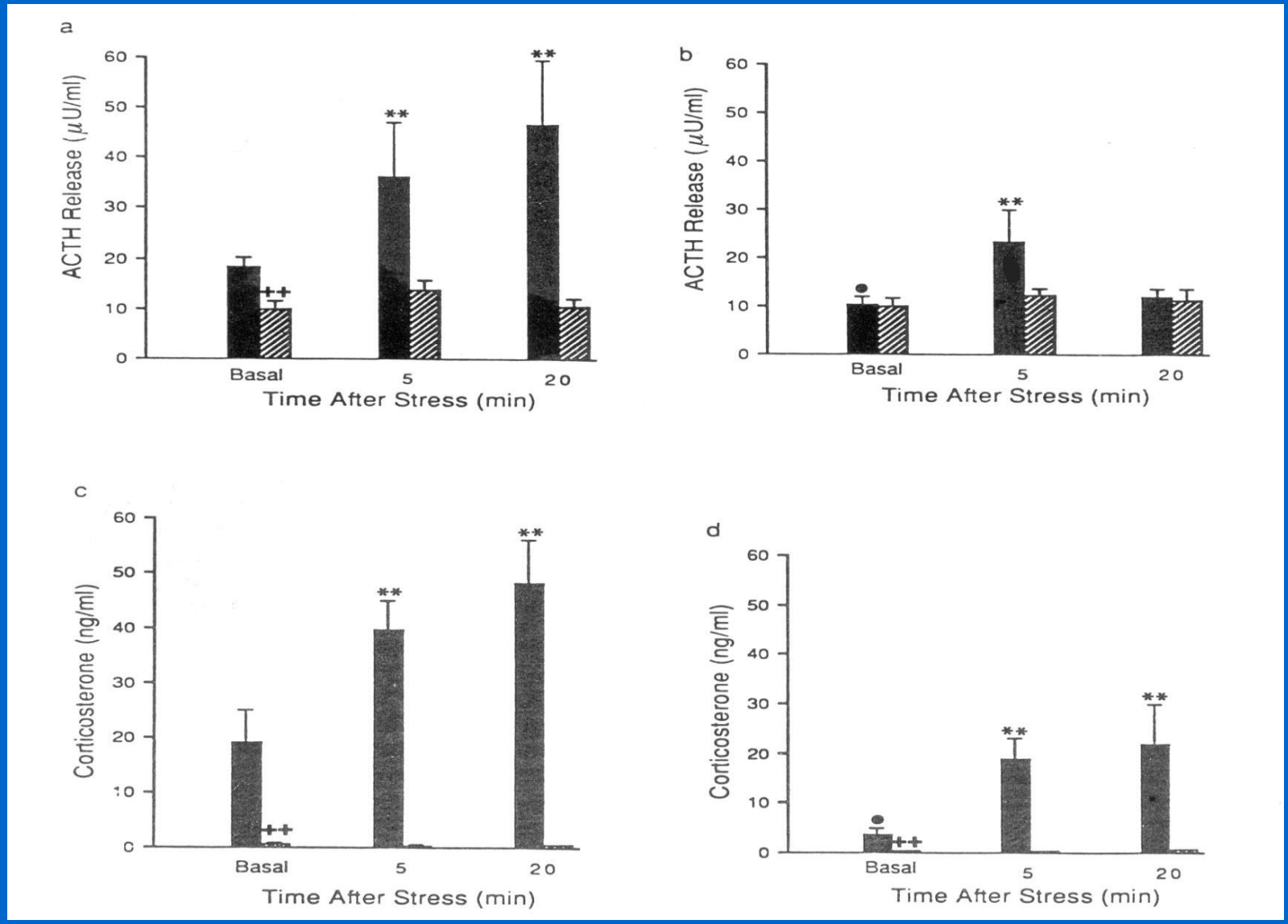
c) GABA

- neurons in mPOA synapse with GnRH neurons

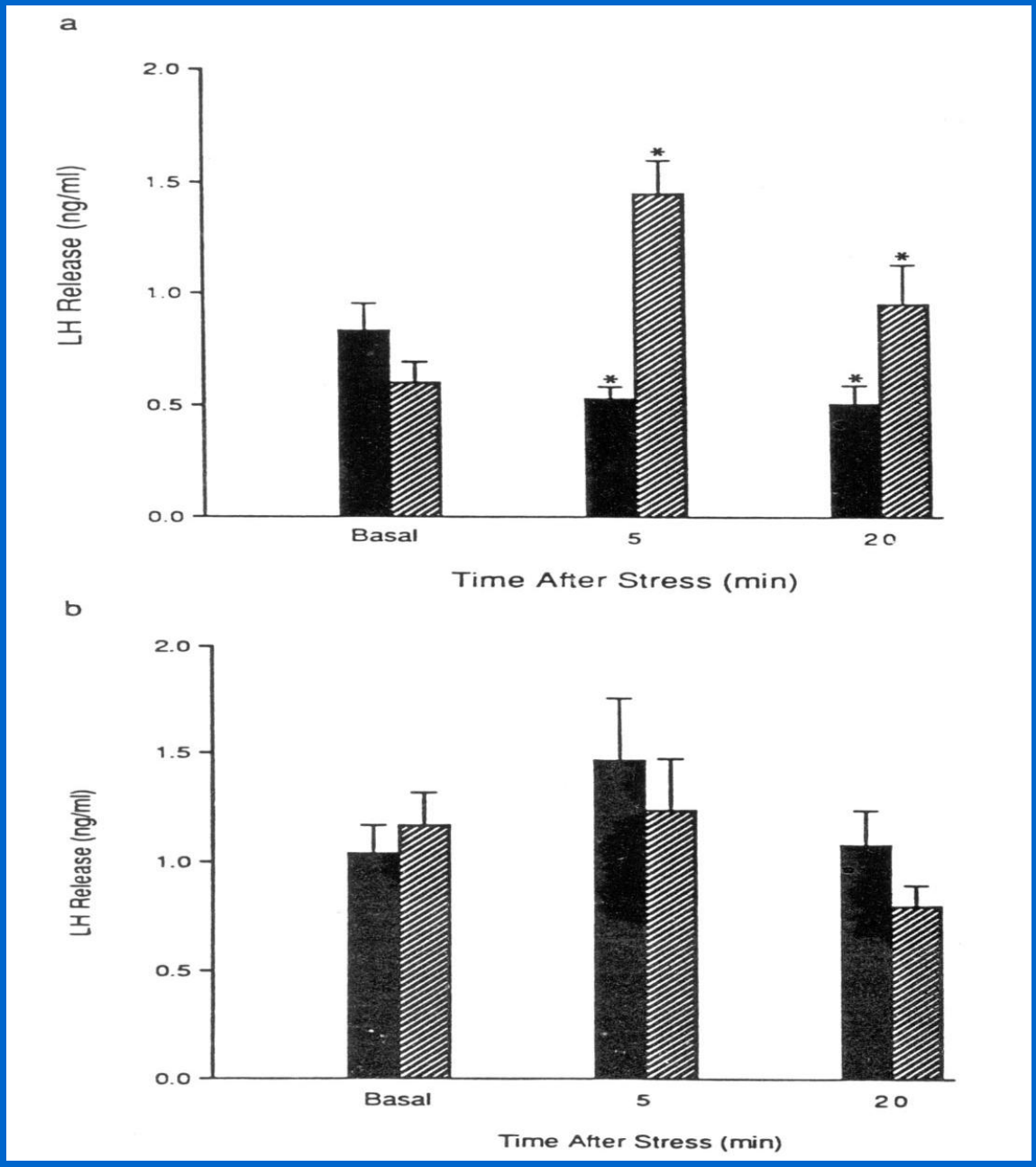
Role of Vasopressin - Brattleboro rat



Effect of stress in Brattleboro and Long Evans rats



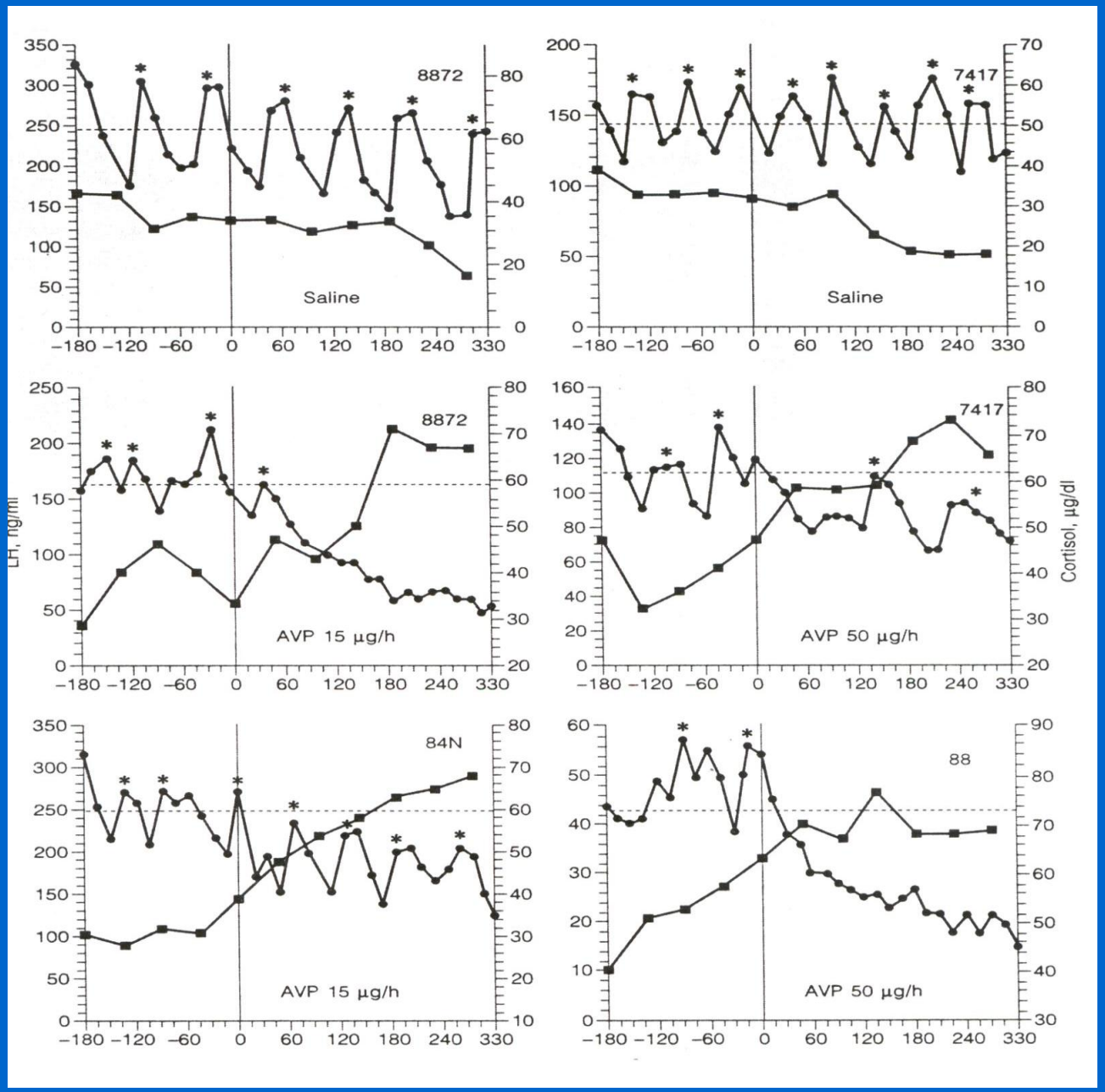
Cover et al 1991


a
LH Release (ng/ml)
2.0
1.5
1.0
0.5
0.0
Basal
5
20
Time After Stress (min)
b
LH Release (ng/ml)
2.0
1.5
1.0
0.5
0.0
Basal
5
20
Time After Stress (min)

- **Thus AVP is essential for the full expression of adrenocortical function.**

- **The Brattleboro rat exhibits a different LH response to stress, suggesting that AVP may contribute to the histamine-induced suppression of LH secretion.**

Effect of AVP on LH secretion



Shalts, E. et al. 1994

Site of action of AVP

Hypothalamus

- AVP does not affect the secretion of LH by isolated pituitary tissue *in vitro*.
- Direct effects on the GnRH neuron – synapses between AVP and GnRH-ir neurons demonstrated.
- Via other neuronal systems
  - neurons containing AVP in close apposition with β-endorphinergic neurons within the hypothalamus.
  - AVP can stimulate the release of beta-endorphin from hypothalamic tissue *in vitro* and *in vivo*.

Glucocorticoids

1. Depressed serum levels of LH commonly occur in subjects of either sex with Cushing's syndrome.

2. Similarly reduced LH in subjects undergoing chronic glucocorticoid therapy

   (An inhibitory action of CRH and AVP in this circumstance is unlikely because exogenous glucocorticoids suppress CRH and AVP secretion).

Evidence of glucocorticoid induced inhibition of HPG activities

Glucocorticoid treatment:-

- Reduces LH secretion
- Reduces GnRH-stimulated LH release
- Results in a failure of ovulation
- Implanted in the MBH prevents the normal onset of puberty

Glucocorticoid site of action

Hypothalamus

- cortisol implanted into MBH prevents normal onset of puberty
- Injection of dex into POA inhibits ovulation
- Glucocorticoids inhibit electrical activity of MBH neurons

Mechanism

- GnRH gene contains corticosteroid regulatory element.
- Non genomic mechanism (<30mins)
- GR co-localised in most neurons in hypothalamus

Pituitary

In vivo: inhibits LH surge
causes a decrease in responsiveness to exogenous GnRH

In vitro: preliminary results suggest inhibition of the ‘priming’effet of GnRH
conflicting data –

Mechanism

GR expressed in gonadotrophs as well as other pituitary cell types.
GR on folliculostellate cells - production of anxa1.
Anxa1R on gonadotroph cell – paracrine effects

Ovary

- Dex inhibits FSH-induced aromatase activity in cultured cells granulosa cells
- Granulosa cells possess glucocorticoid receptors

Testes

- GR receptors localised in interstitial Leydig cells
- ↓ testicular LH receptor concentration
- ↓ -stimulated testosterone production

Conclusion

Evidence to suggest that stress can cause a disruption of reproduction.

The way that this comes about remains to be clarified however the hormones of the HPA axis (CRH, AVP and glucocorticoids) can exert inhibitory effects at all levels of the HPG axis.

The Facilitation of LH release

- Acute stress ↑ LH secretion in oestrogen-primed rats
- Similar results in OVX + E2 monkey
- Menopausal women receiving oestrogen replacement, endotoxin administration ↑ LH

Acute stress affects the length of follicular and luteal phases of the cycle

Gonadal steroids environment has an important role in determining the effect observed.

Does facilitation of LH secretion fit into our scheme?

An acute stress, too small to interrupt the reproductive cycle, may still interfere with cycle function.

Elevated LH concentrations in mid to late follicular phase may damage the maturing follicle/and or oocyte.

In conclusion

- Activation of the HPA axis has been demonstrated to exert a inhibitory effect on reproductive function (via CRH/AVP, and corticosteroids).

- Stress can also interfere with fertility by facilitating LH secretion (depending on the gonadal steroidal environment).

References

- Knobil, E. (1980). The neuroendocrine control of the menstural cycle. Recent Progress in Hormone Research 36. 53-89.
- Kauffman, A.S. (2004). Emerging functions of gonadotropin-releasing hormone II in mammalian physiology and behaviour. J Neuroendocrinology 16, 794-806.
- Ferrin, M. (1999). Stress and the reproductive cycle (review). J. Clinical Endocrinology & Metabolism, 84, 1768-1774).
- Rivier, C., Rivier, J. & Vale W. (1986). Stress- induced inhibition of reproductive functions: role of endogenous corticotropin-releasing factor. Science, 231, 607-609
- Cover, P.O., Laycock, J.F., Gartside, I.B. & Buckingham, J.C. (1991) A role for vasopressin in the stress-induced inhibition of gonadotrophin secretion: studies in the Brattleboro rat. J. Neuroendocrinology 3, 413-417.
- Shalts, E. *et al.* (1994) Inhibitory effect of arginine vasopressin on luteinising hormone secretion in the ovariectomized rhesus monkey. Neuroendocrinology, 59, 336-342.
- Jeong KH, Jacobson L, Widmaier EP, Majzoub JA. (1999) Normal suppression of the reproductive axis following stress in corticotropin-releasing hormone-deficient mice. Endocrinology. 140(4):1702-8.
- Breen KM & Karsch FJ (2006) New insights regarding glucocorticoids, stress and gonadotropin suppression. Front. Neuroendocrinol. 27, 233-45.
- Oakley et al (2009) Endocrine Reviews, 30,713- 743. Kisspeptin signalling in the brain.
- Li X.F. et al. (2010) The role of the Bed Nucleus of the Stria Terminalis in stress-induced inhibition of pulsatile luteinising hormone secretion in the female rat. J. Neuroendocrinology 23, 3-11.