



如何快速完成 學位論文

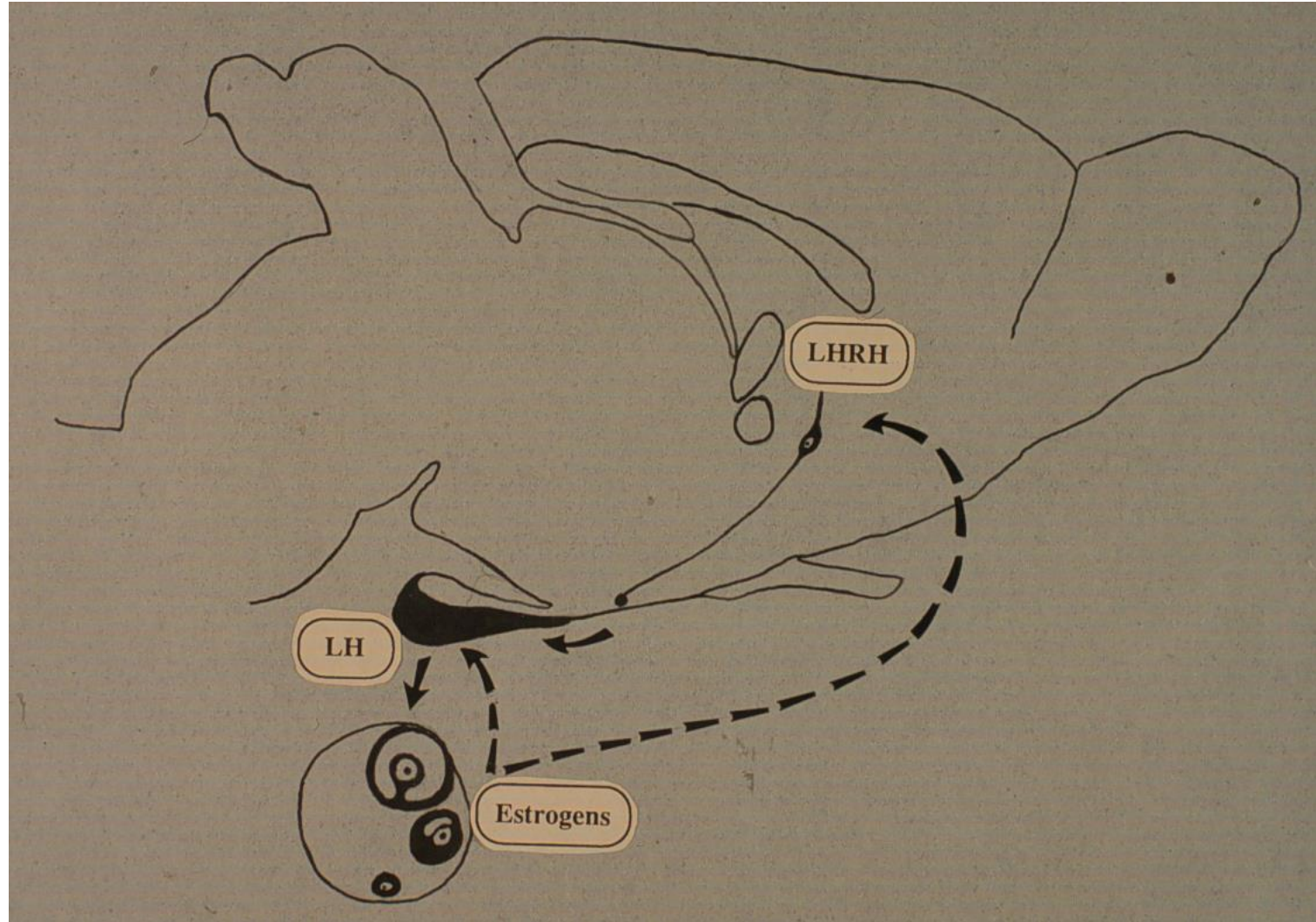
李文森教授

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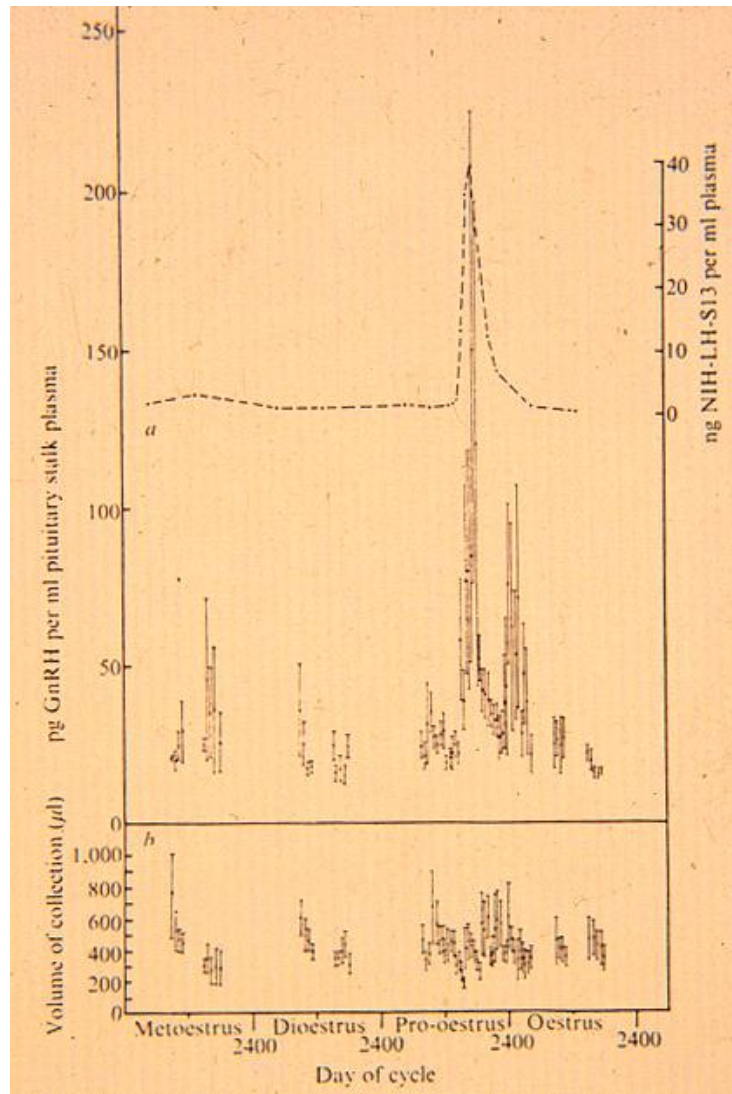
我的博士論文研究

LHRH neuronal activity

Hypothalamus-Pituitary-Gonadal Axis

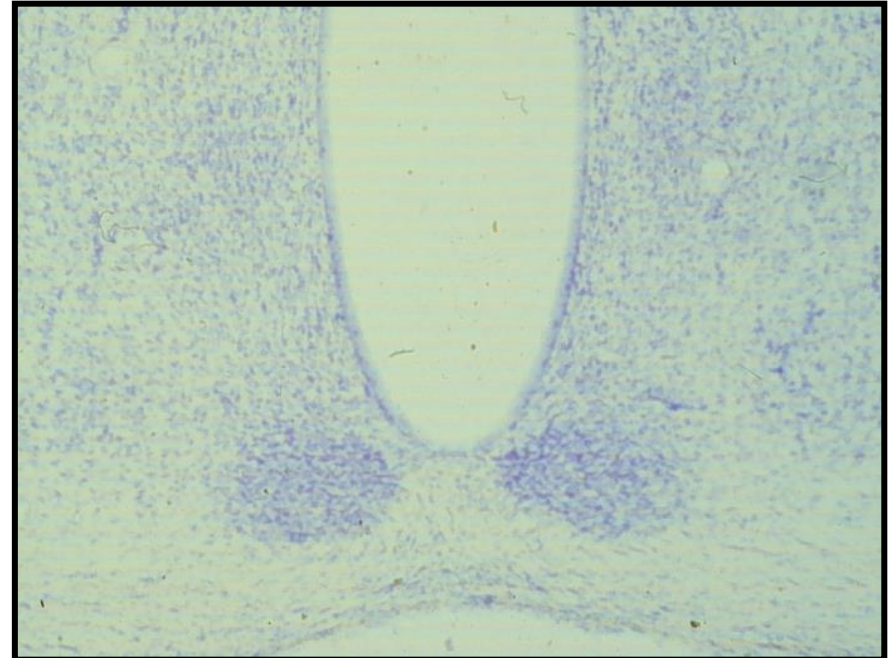
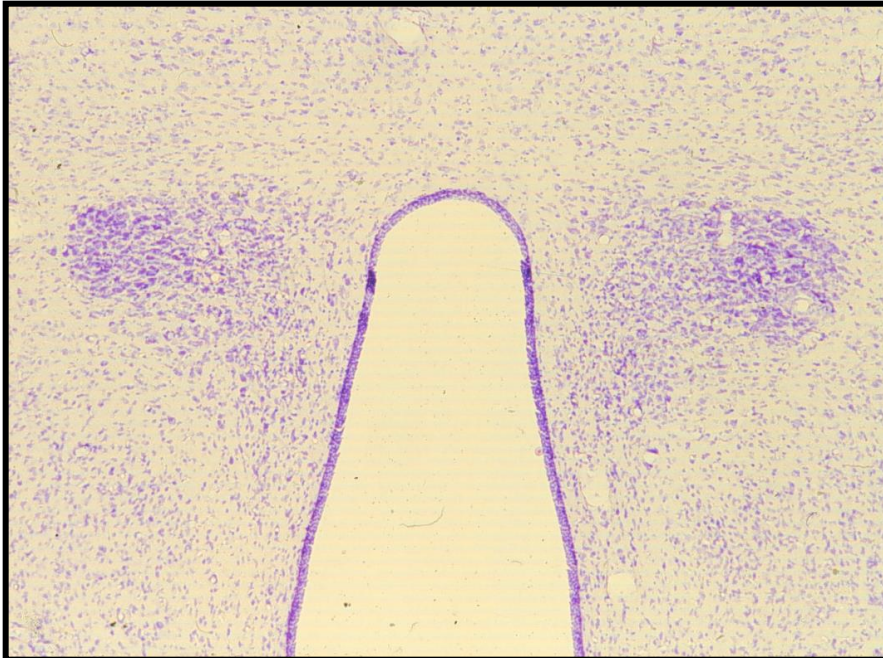


為何要研究LHRH neuronal activity?



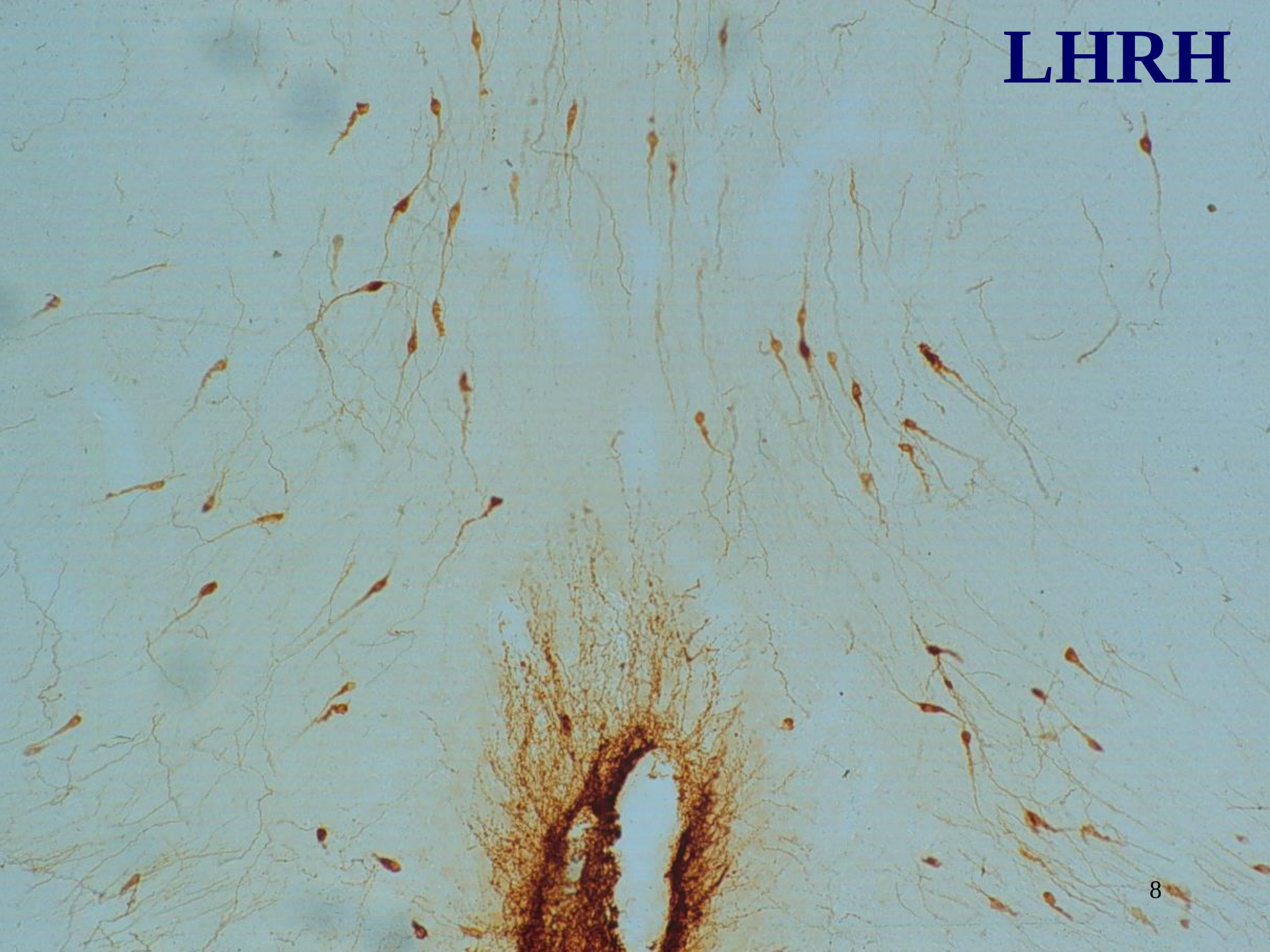
What is the difficulty to study the LHRH activity?

Number of LHRH neurons?



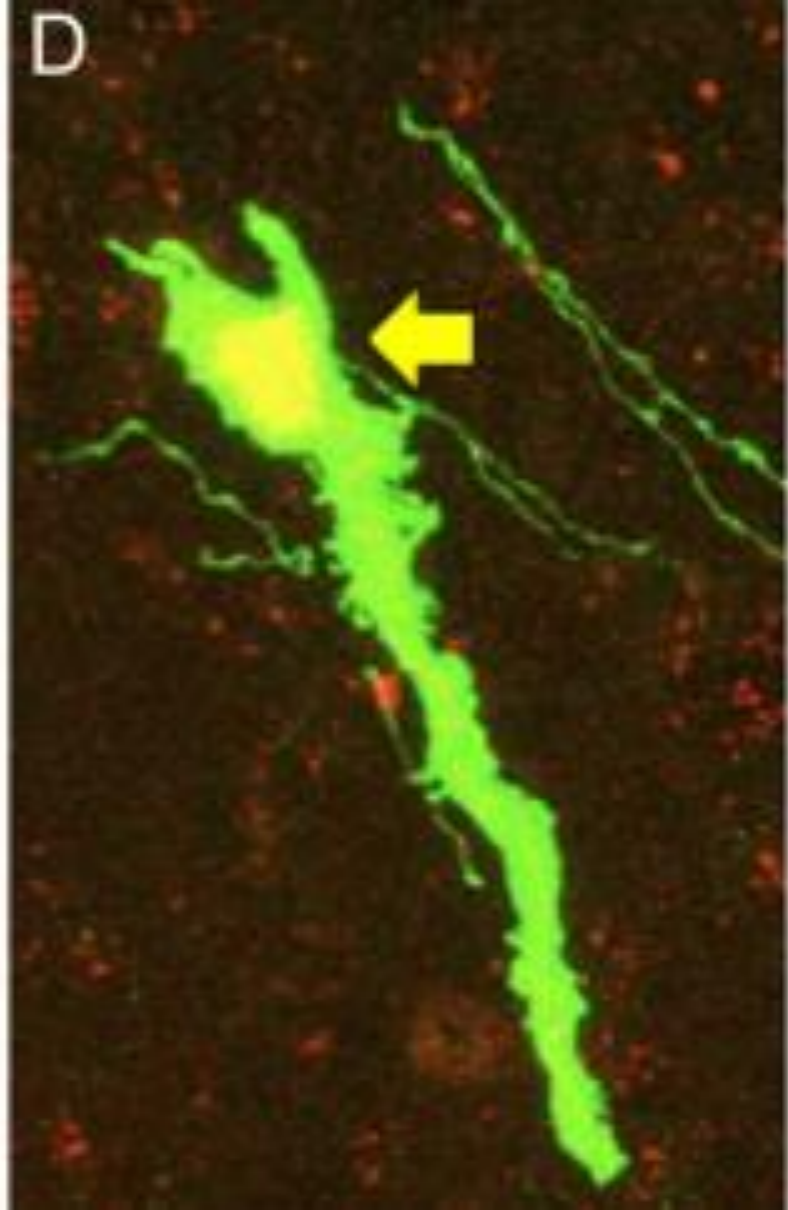
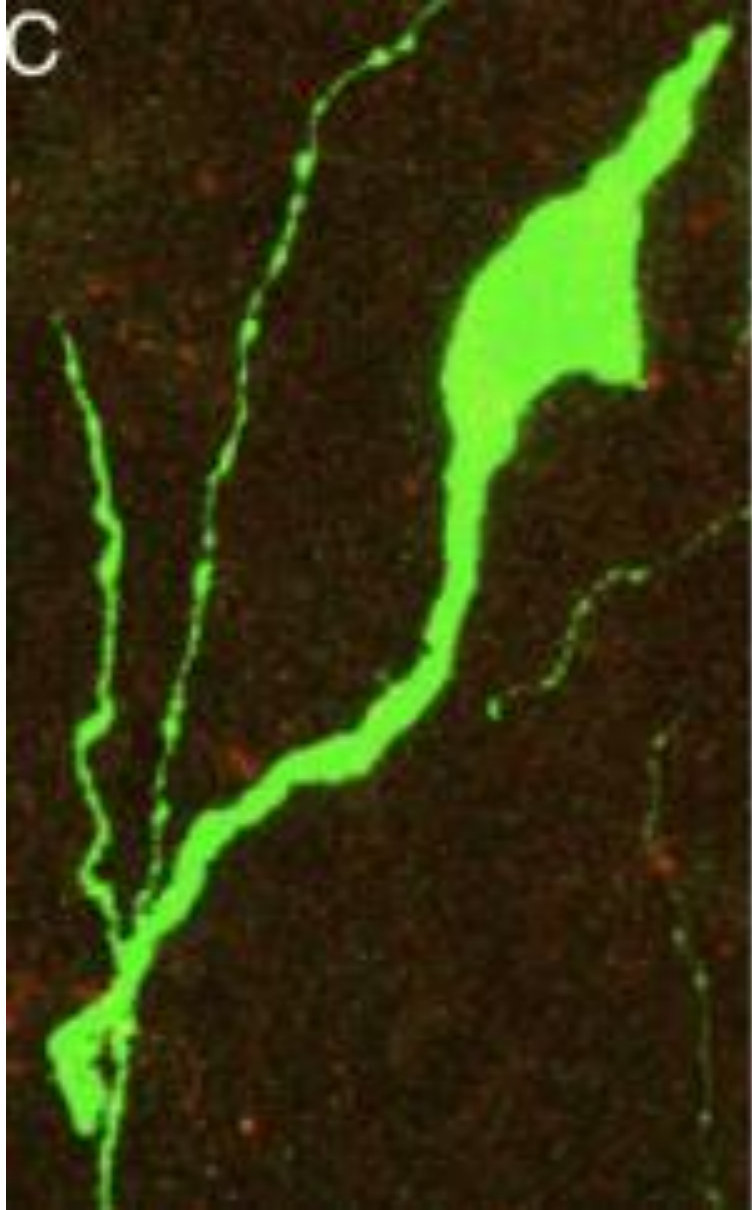
Distribution of LHRH neurons

LHRH



Methods for the LHRH activity evaluation

- **Portal blood collection**
- **Push-pull technique**
- **Multiple or single unit activity**
- **LH secretion**

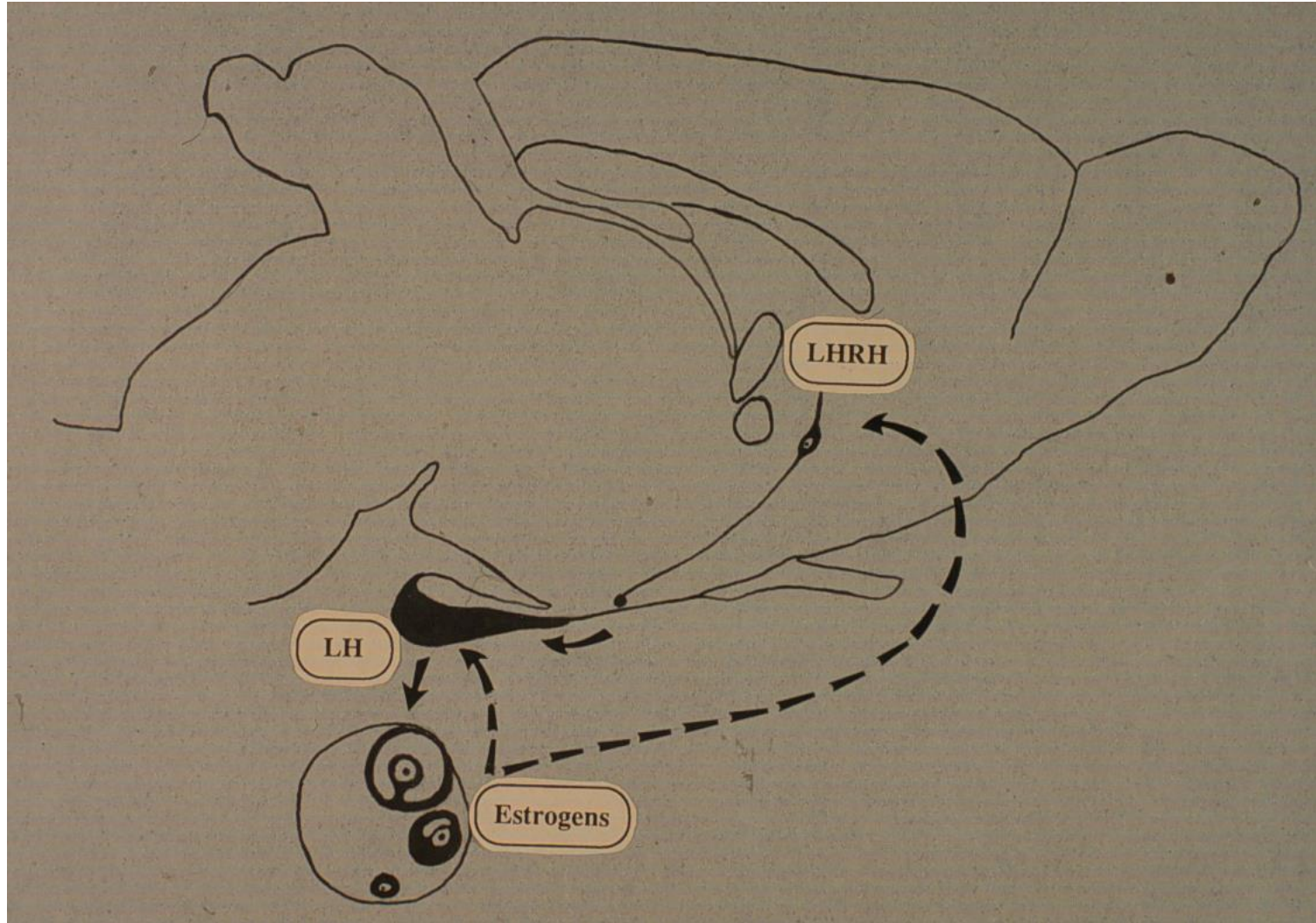


Chan et al., Neuroendocrinology 2011, 152: 4906-4914

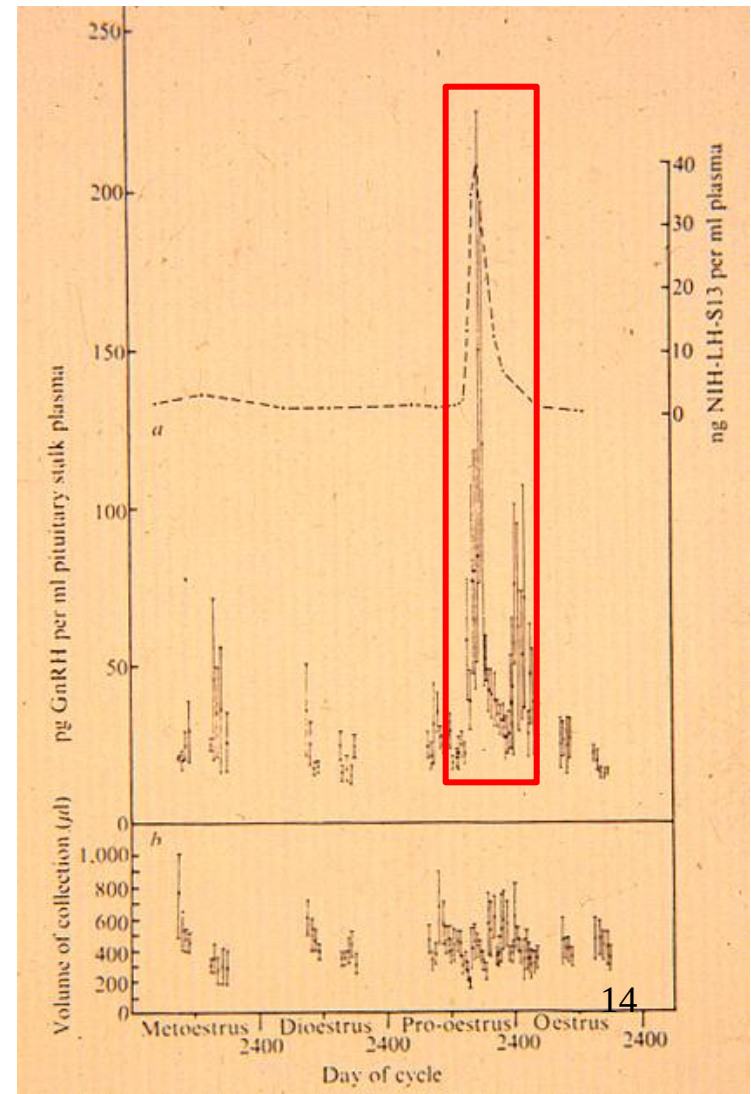
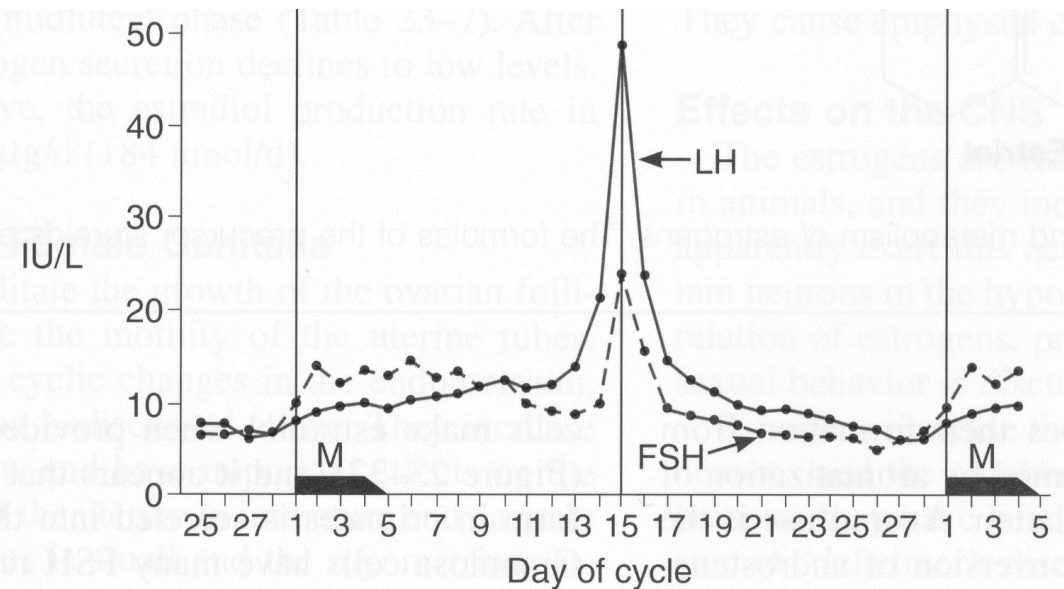
Use of cFos as a marker for LHRH neuronal activation

Ovariectomy

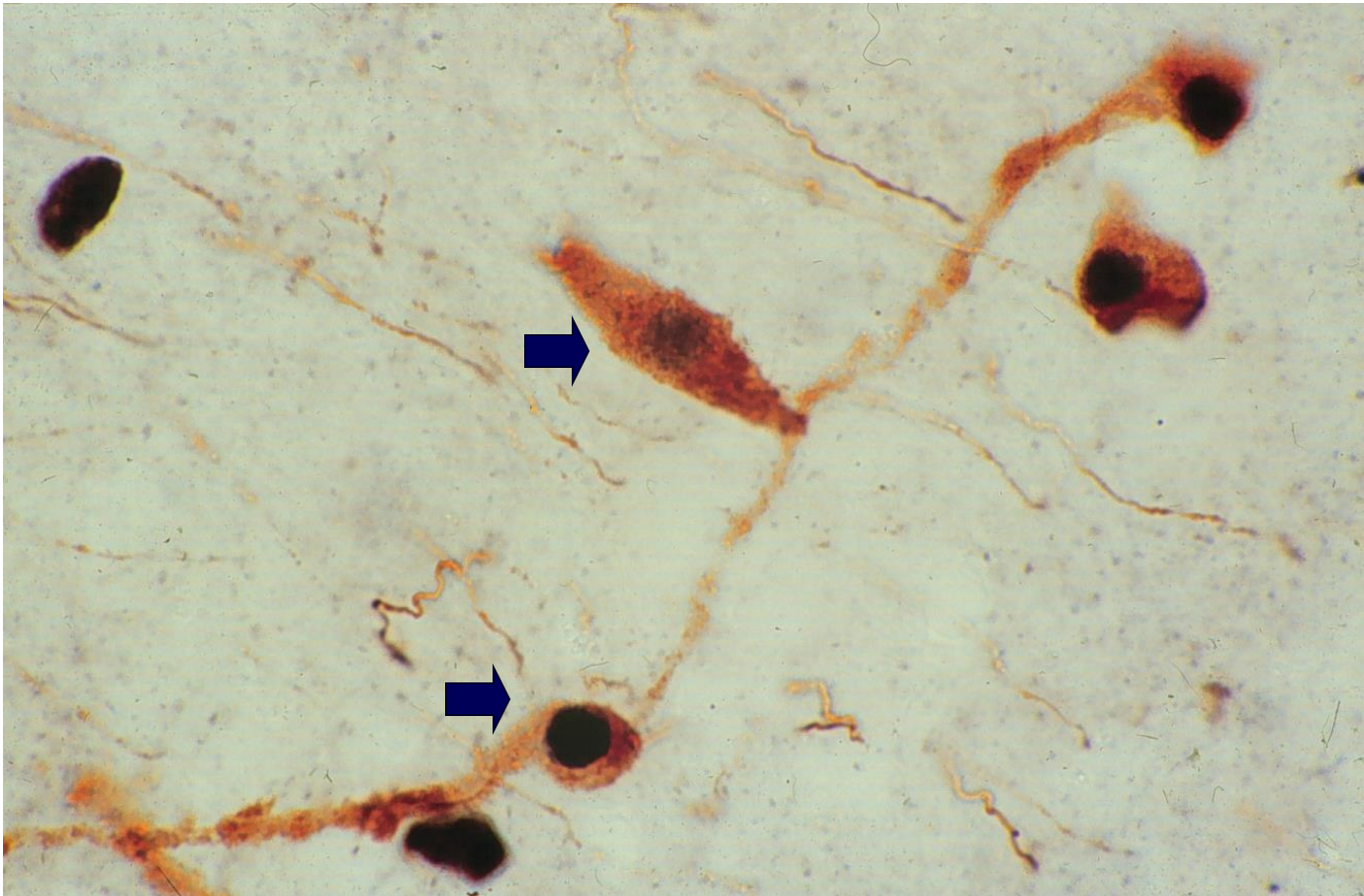
Hypothalamus-Pituitary-Gonadal Axis



Hormonal secretion during menstrual cycle



Expression of cFos in LHRH neurons on the afternoon of proestrus



LHRH neurons do not express cFos in the morning of proestrus

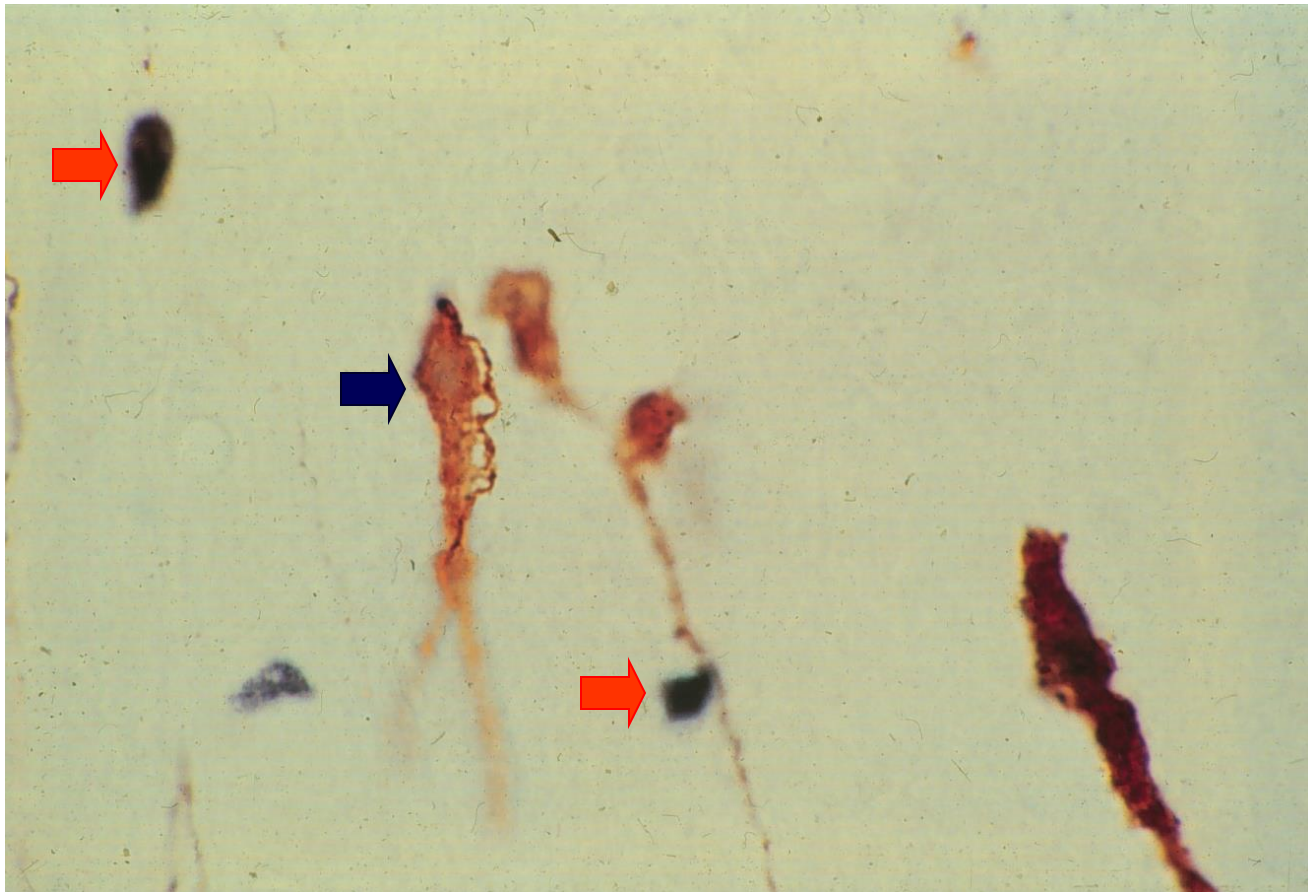
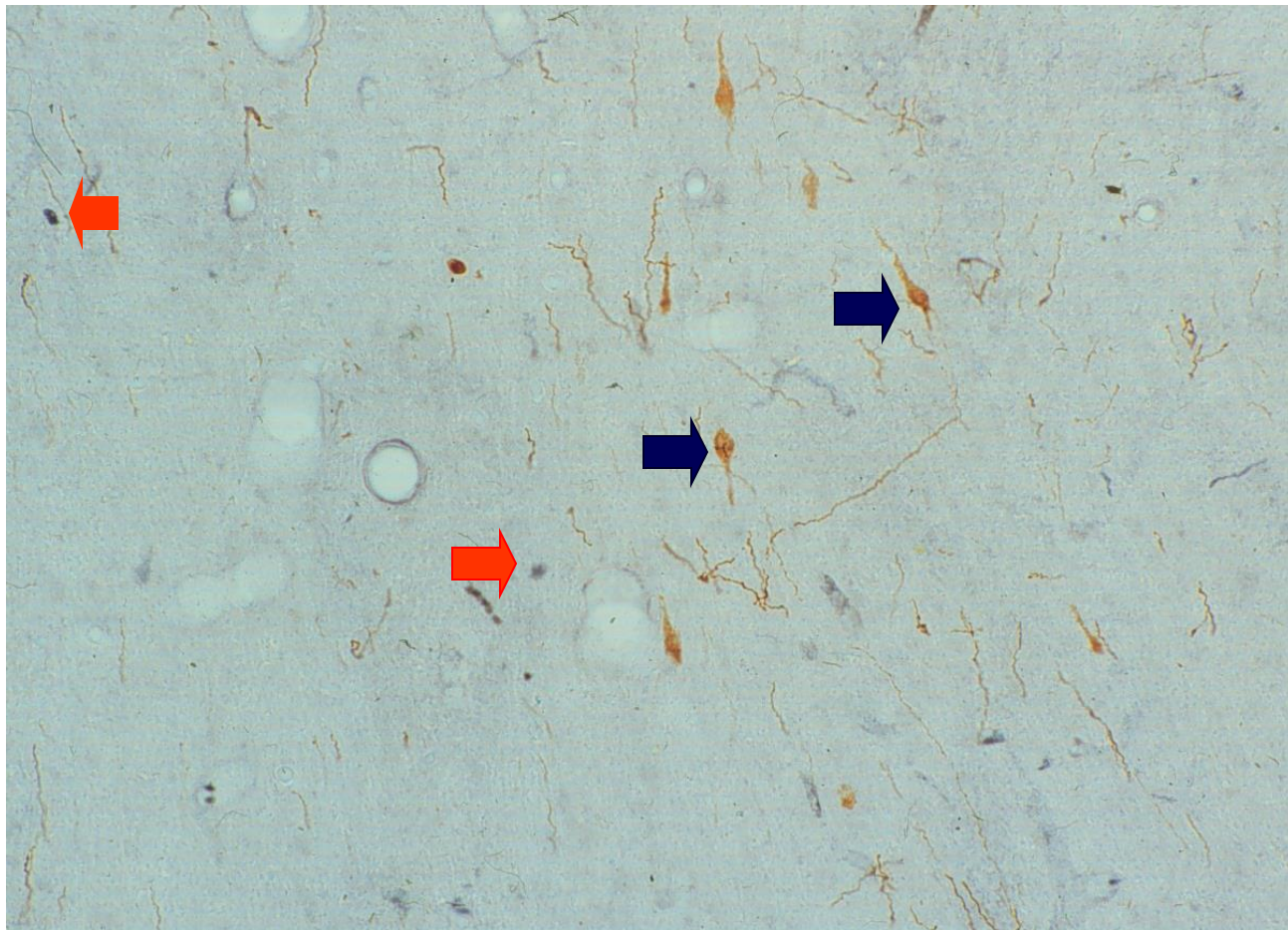


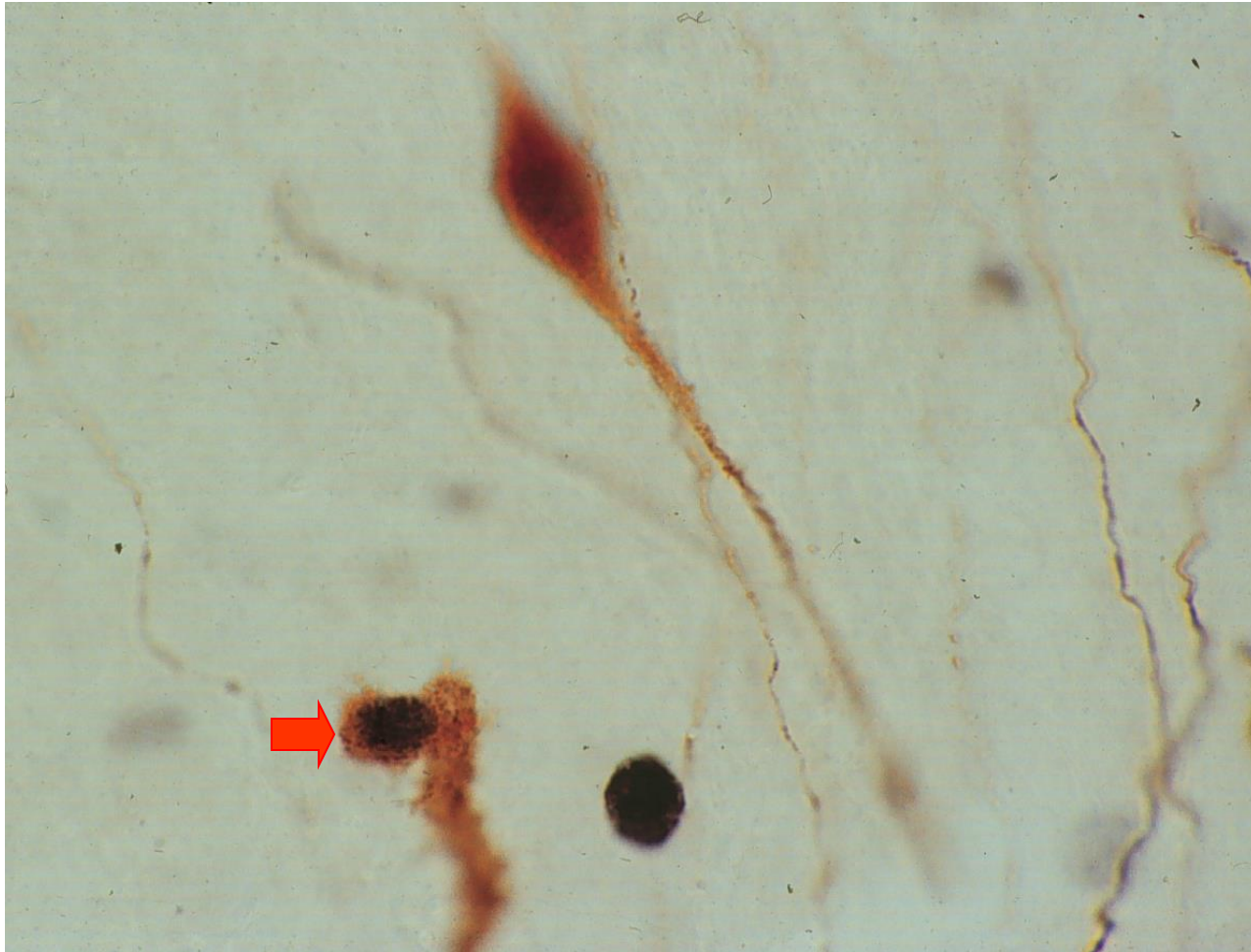
Table 1. Fos expression in LHRH neurons during the estrous cycle

Day of sacrifice	Time	<i>n</i>	Fos staining
Diestrus-1	0900–1100	3	—
	1600–1700	3	—
Diestrus-2	0900–1100	3	—
	1600–1700	3	—
Proestrus	0900–1100	3	—
	1400–1530	6	—
	1530–1700	4	+
	1700–1800	4	+
	1800–2200	4	+
Estrus	0900–1100	3	—
	1600–1700	3	—

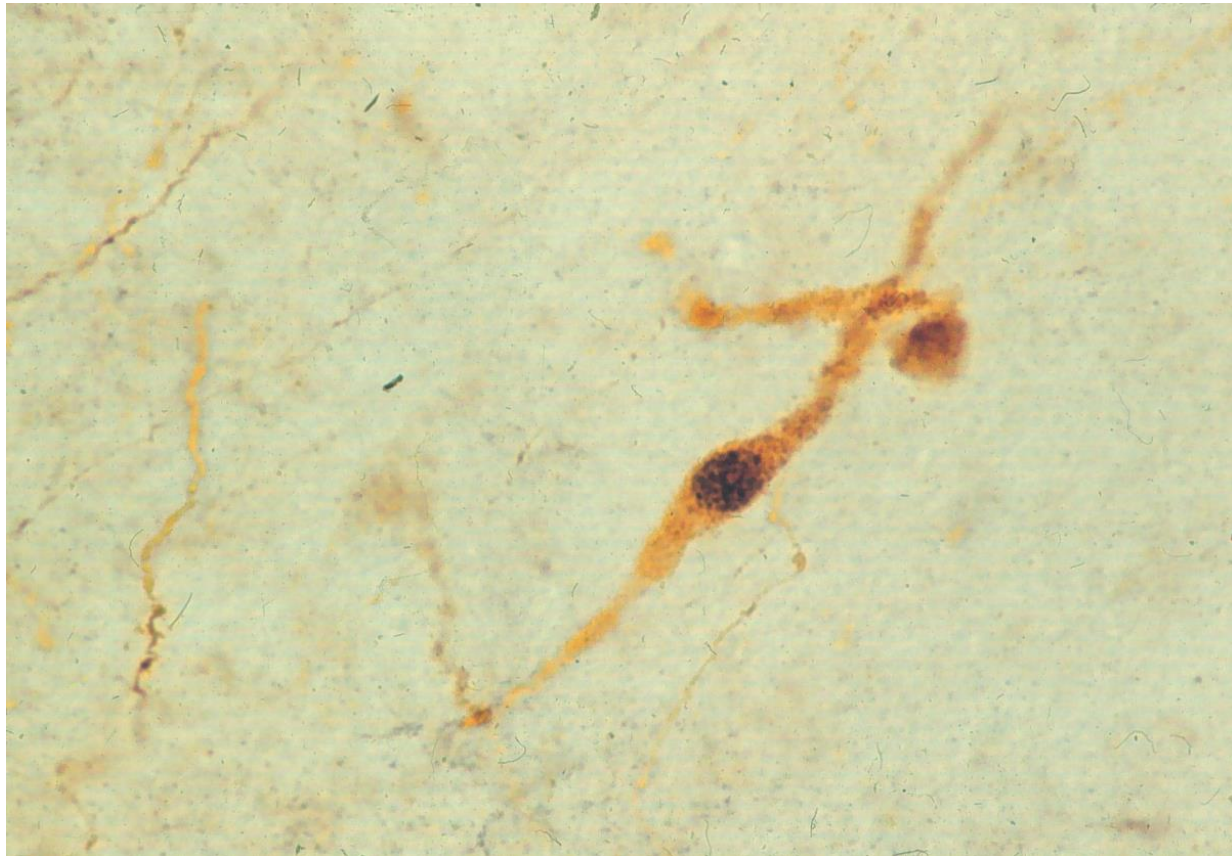
Pentobarbital blocks the expression of cFos in LHRH neurons on the afternoon of proestrus



Delayed expression of cFos after pentobarbital treatment



Estradiol induces cFos expression in LHRH neurons of the rat at age 28



**cFos could serve as a
marker for LHRH
neuronal activation**

1990/1/1-1991/7/31

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先確定自己研究的end point

科學三要素

- 觀察
- 假說
- 實驗

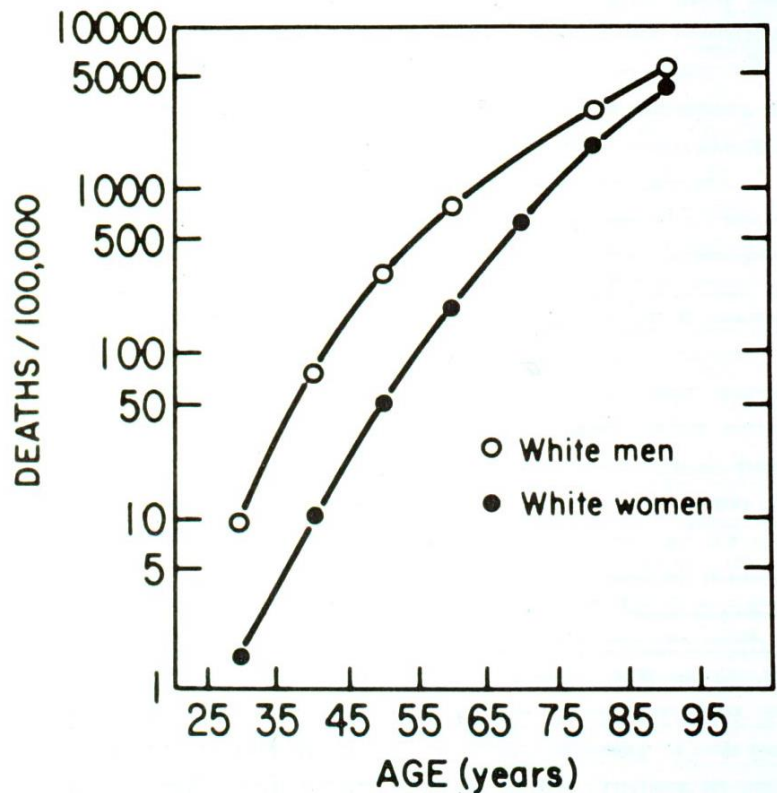
建立研究的Hypothesis

如何建立 **Hypothesis**

要有充分的 **previous findings**

Rationale

Epidemiological study



Furman RH Ann NY Acad. Sci. 1968

Pre-menopausal women have a much lower mortality from atherosclerotic cardiovascular disease than men. However, during their postmenopausal years, cardiovascular disease in women gradually approaches that of men.

Hypothesis

Female sex hormones may have a cardiovascular protective effect in pre-menopausal women.

Previous findings

From literatures

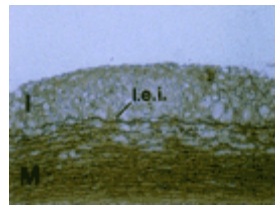
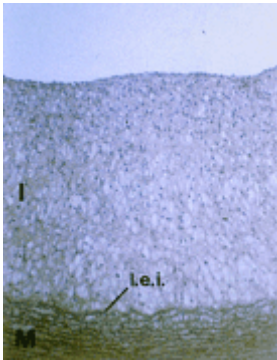
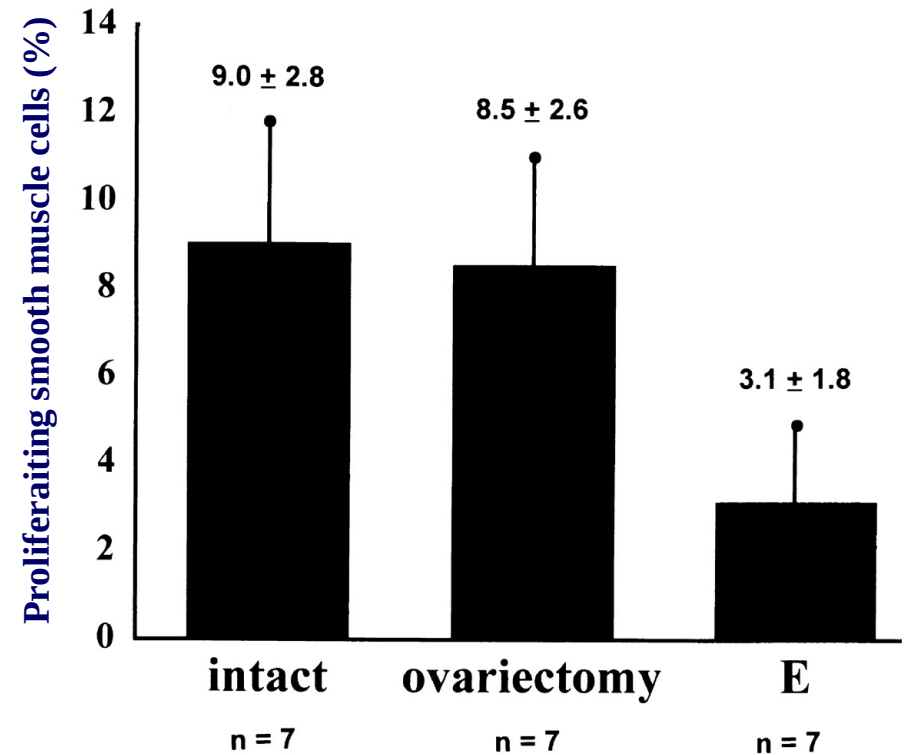
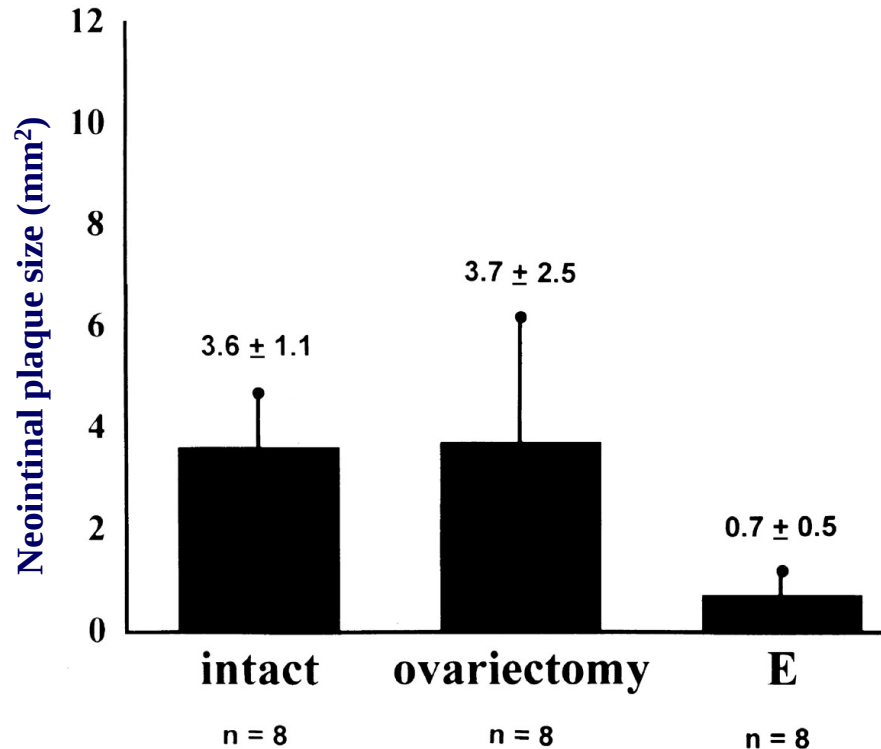
From your own or labmates

Supporting evidence

Estrogen replacement reduces the incidence of cardiovascular diseases in the postmenopausal women

Stampfer MJ et al., N Engl J Med. 1991 325(11):756-62.

Effect of estrogen on experimental atherosclerosis in female rats



Hanke H et al. Circulation 94:175-181, 1996

Effect of 18 months of hormone therapy on plasma cholesterol and lipoprotein cholesterol concentrations in the baboon

Treatment	n	Plasma cholesterol (mg/dl)	VLDL+LDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL+LDL/HDL cholesterol (ratio)
Control	6	161 (133-195)	77 (58-101)	80 (62-104)	0.95 (0.54-1.40)
Estrogen	6	144 (129-160)	55* (47-66)	87 (76-99)	0.63* (0.52-0.63)

Kushwaha et al., Arterioscler Thromb. 11:23-31, 1991

需要多少證據來支持
你的假說

Progesterone can directly affect the proliferation of vascular smooth muscle cells

- 1. The presence of progesterone receptor in the vascular smooth muscle cells.**
- 2. Progesterone can affect the proliferation of vascular smooth muscle cells.**
- 3. Progesterone regulates the proliferation of vascular smooth muscle cells through the progesterone receptor-mediated pathway.**

Presence of progesterone receptor in the vascular smooth muscle cells

- 1. Western blot analysis**
- 2. Immunocytochemical staining**
- 3. RT-PCR**
- 4. In situ hybridization**

Progesterone can affect the proliferation of vascular smooth muscle cells

1. Cell count

2. MTT assay

3. Flow cytometric analysis

Progesterone regulates the proliferation of vascular smooth muscle cells through the progesterone receptor-mediated pathway

- **Blockade of progesterone activity can abolish the progesterone effect on vascular smooth muscle cells.**
- **Knockout progesterone receptor can abolish the progesterone effect on vascular smooth muscle cells.**

**Molecular mechanisms underlying
progesterone affect the proliferation of
vascular smooth muscle cells**

Call for collaborators

Right model

**Interdisciplinary
techniques**

Magic fingers

了解每一實驗步驟的意義

時間管理

進實驗室前先熟悉
整個實驗流程

可以同步進行多項平行實驗

不要讓一個實驗卡住了整個研究

工作日記

請指導教授盡早幫你組成 thesis committee

Committee members的工作不只是在考核你的研究能力與成果，更重要的是幫助你順利完成學位論文

論文題目確定後就可以
以邊進行實驗邊著手
撰寫論文的overview

文章雖可分開發表但整本
論文是一個連貫的故事

Epilogue

要找出研究的樂趣

快樂比較容易成功

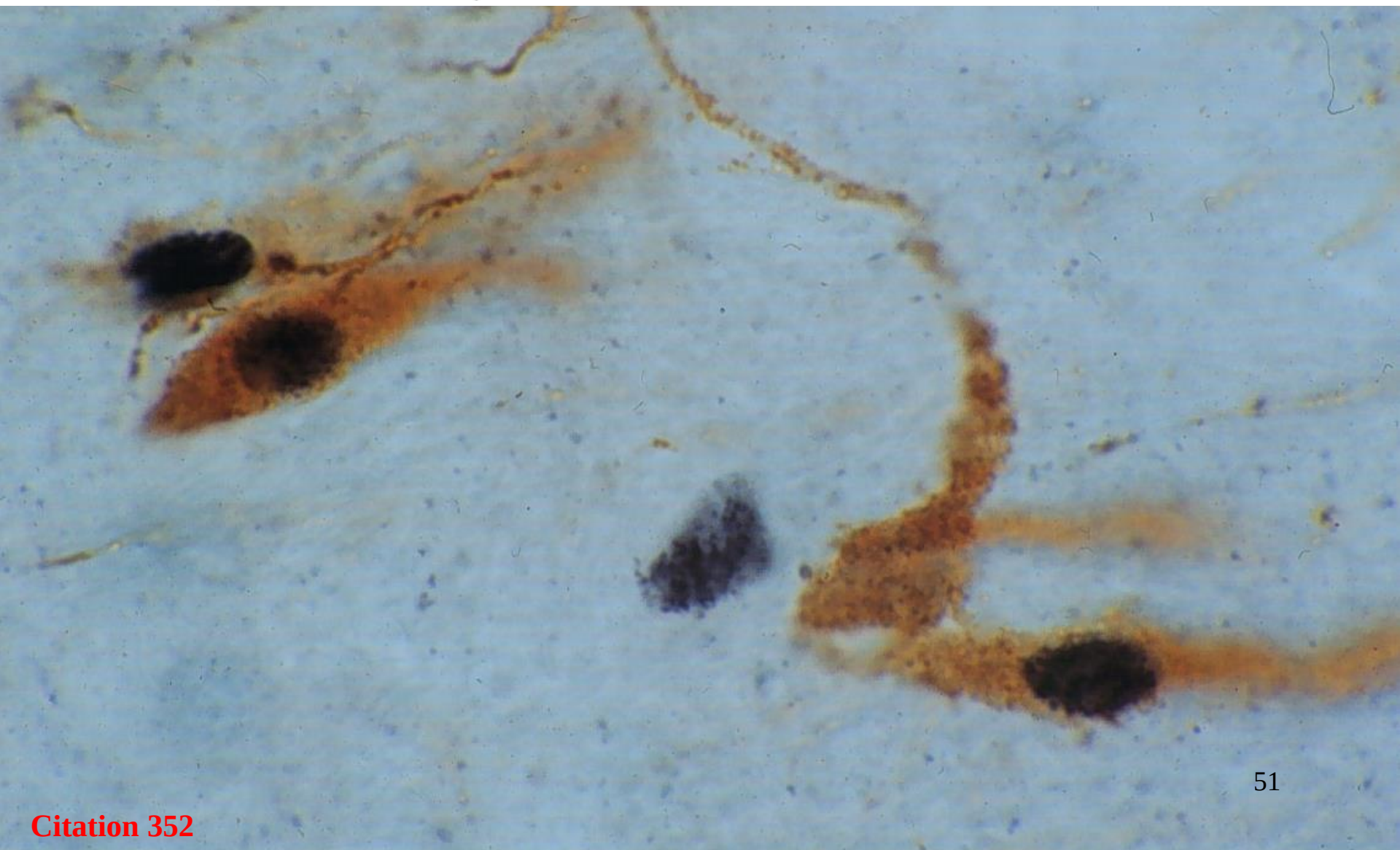
研究的最大動力？

Reward

Luteinizing hormone-releasing hormone neurons express Fos protein during the proestrous surge of luteinizing hormone

(oncogene product/estrous cycle/immunocytochemistry/hypothalamus/preoptic area)

WEN-SEN LEE, M. SUSAN SMITH, AND GLORIA E. HOFFMAN*



Team work

1991/8/1-1994/7/31

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