

B 3582



THESES

**ANATOMICAL RELATIONSHIP BETWEEN THE BIOLOGICAL
CLOCK AND THE NEUROENDOCRINE HYPOTHALAMUS**

Dr. Tamás L. Horváth

**Department of Obstetrics and Gynecology
Yale University School of Medicine**

**NEW HAVEN
1998**

THESES

**ANATOMICAL RELATIONSHIP BETWEEN THE BIOLOGICAL
CLOCK AND THE NEUROENDOCRINE HYPOTHALAMUS**

Dr. Tamás L. Horváth

**Department of Obstetrics and Gynecology
Yale University School of Medicine**

**NEW HAVEN
1998**

INTRODUCTION

The biological clock located in the hypothalamic suprachiasmatic nucleus (SCN) in conjunction with the intergeniculate leaflet of the lateral geniculate body (IGL) provide circadian and visual signals for the temporal organization of endocrine and autonomic mechanisms supporting higher brain functions. One component of clock-driven endocrine mechanisms is the circadian gonadotropin and lactotrop hormone secretions from the anterior pituitary that is pivotal for the maintenance of normal reproduction in all species thus far studied.

The pituitary secretion of gonadotrop hormones, luteinizing hormone releasing hormone (LH) and follicle stimulating hormone (FSH), and the lactotrop hormone, prolactin are under the regulation of the hypothalamus. In the hypothalamus, humoral signals arising from the gonads (testosterone, estradiol and progesterone) and neuronal signals arising from the circadian clock are integrated to regulate the final output neurons of the hypothalamus underlying pituitary gonadotropin and prolactin secretions, the gonadotropin releasing hormone (GnRH)- and dopamine-producing neural circuits. The circadian activity of the rat hypothalamo-pituitary axis is gender specific, only females having the ability to manifest surges of gonadotropin and prolactin releases in response to elevating estradiol and progesterone levels and adequate circadian signals. While a large body of morphological and physiological evidence has accumulated to explain these gender specific endocrine mechanisms, the signaling pathway from the circadian clock to neuroendocrine cells is ill defined. It is also not known whether the gender specific nature of pituitary hormone secretions may be supported by sex differences in the circadian clock and whether the integration of hormonal signals into the hypothalamo-pituitary axis may occur outside of the hypothalamus in part of the extended biological clock. Experiments in this thesis were designed to fill these *hiata* and, thus, gain further insights into the central regulation of anterior pituitary hormone secretions.

OBJECTIVES

The following specific objectives were to be tested:

- 1) Does the circadian clock, SCN, provides direct signals for neuroendocrine cells, including those producing GnRH and dopamine?
- 2) Can the IGL in the lateral geniculate body provide signals to neuroendocrine cells independent of the SCN?
- 3) Can gonadal signals be integrated into the hypothalamo-pituitary axis outside of the hypothalamus, in the IGL?
- 4) Is the development of the biological clock under the control of gonadal steroids?
- 5) Is the SCN input to GnRH cells gender specific?

MATERIALS AND METHODS

OBJECTIVE 1) Does the circadian clock, SCN, provides direct signals for neuroendocrine cells, including those producing GnRH and dopamine? A combination of anterograde, retrograde tracing and light and electron microscopic multiple label immunocytochemistry was carried out.

OBJECTIVE 2) Can the IGL in the lateral geniculate body provide signals to neuroendocrine cells independent of the SCN? In addition to anterograde, retrograde tracing and light and electron microscopic multiple label immunocytochemistry, this study also employed acute axonal degeneration to label efferents of the retina.

OBJECTIVE 3) Can gonadal signals be integrated into the hypothalamo-pituitary axis outside of the hypothalamus, in the IGL? To test this hypothesis, in situ hybridization histochemistry was carried out for estrogen receptor beta (ER β) and progesterone receptor (PR).

OBJECTIVE 4) Is the development of the biological clock under the control of gonadal steroids? To test this hypothesis, in this experiment, immunocytochemistry for aromatase, the key enzyme in sexual differentiation, was carried out on different areas of the developing rat. To confirm the existence of aromatase in these areas, a biochemical assay for aromatase activity was also done.

OBJECTIVE 5) Is the SCN input to GnRH cells gender specific? To address this issue, quantitative analysis of the vasoactive intestinal polypeptide (VIP)-containing input of GnRH neurons was assessed in male and female rats. This input has previously been shown to originate in the SCN.

RESULTS

1. The circadian clock, SCN, provides direct signals for neuroendocrine cells, including those producing GNRH and dopamine (Objective 1)

This study using anterograde and retrograde tracing techniques in combination with immunocytochemistry, provided evidence that: 1) the circadian pacemaker suprachiasmatic nucleus send direct efferents onto neuroendocrine cells of different hypothalamic nuclei, 2) a subpopulation of SCN target neuroendocrine cells contains dopamine and GnRH, and 3) suprachiasmatic efferents do not reach fenestrated capillaries. These observations suggest that the circadian pacemaker has no direct effect on the regulation of anterior pituitary functions, but, indicate a pathway via circadian signals are integrated into the hypothalamo-pituitary-gonadal axis. It needs to be explored whether the integration of hormonal and circadian signals, a mandatory process in the regulation of anterior pituitary, which was indicated to occur in populations of hypothalamic neurons (158) may be the same cells that were found to be neuroendocrine in the present study.

2. The IGL in the lateral geniculate body can provide signals to neuroendocrine cells independent of the SCN (Objective 2)

These experiments revealed that a population of LGN-targeted neurons in the hypothalamus are neuroendocrine cells, i.e., they have direct access to the portal vasculature of the median eminence or the organum vasculosum laminae terminalis. These cells, including those producing dopamine, were most frequently found in periventricular areas. The same hypothalamic cell populations were found to receive SCN input in Objective 1, raising the possibility of convergent SCN and IGL inputs on the same hypothalamic perikarya. In light of the fact that the parent cells of the IGL efferents were found to receive direct visual input, it is reasonable to suggest that the integration of visual and circadian signals into the hypothalamo-pituitary axis may occur on the final output neurons of the hypothalamus adding another level of redundancy to the pathways via which the environment may regulate hormone secretions.

3. Gonadal signals can be integrated into the hypothalamo-pituitary axis outside of the hypothalamus, in the IGL (Objective 3)

This study provided evidence for the expression of ER- β and PR mRNA in the ventral LGN and IGL using in situ hybridization histochemistry. The riboprobes used in the present study have been well characterized and shown to be specific for the transcripts these gonadal steroid receptor genes.

The amount of ER- β and PR transcripts present in vLGN and IGL cells seemed to be lower compared to other limbic and hypothalamic regions where the abundance of silver grains over cells was observed to be much higher. A comparative analysis of mRNA labeling intensity within the rat brain has been reported for ER- β . In that study, while the presence of ER- β mRNA has been mentioned in the vLGN, the IGL was not analyzed separately and no quantitation was given in regard to the size of the neuronal population within this thalamic region expressing ER- β mRNA.

4. The development of the biological clock is under the control of gonadal steroids (Objective 4)

This study clearly demonstrated that both components of the extended biological clock, the SCN and IGL, are sites of local estrogen production during the critical developmental period. This observation raise the possibility that sexual dimorphisms in the extended biological clock may exist and support the gender specific regulation of anterior pituitary hormones. Part of this hypothesis was tested in Objective 5, when the SCN-derived VIP innervation of GnRH neurons was assessed in male and female rats.

5. The SCN input to GnRH cells is gender specific (Objective 5)

This study provided light microscopic evidence for a sexual difference in the percentage of GnRH-synthesizing cells that receive VIP input. VIP-immunoreactive axons regularly showed interaction with GnRH neurons in both males and females. Yet, we found significantly more VIP-GnRH interaction in females ($34.5 \pm 4.1\%$) than in males ($17.3 \pm 2.1\%$; $p < 0.001$). Also, the frequency of VIP contacts on individual GnRH neurons was significantly higher in females (2.6 ± 0.23) as compared with males (1.3 ± 0.15 ; $p < 0.001$).

DISCUSSION

The circadian/visual system is a phylogenetically preserved system that allows for the temporal organization of the environment and the organism. In this thesis we carried our experiments that provide new insights into a particular output of the extended biological clock, i.e., the rhythmic regulation of the hypothalamo-pituitary axis. We demonstrated alternate routes of signaling from the circadian clock, SCN, and eye to the neuroendocrine hypothalamus. We also revealed that the mandatory integration of hormone signals into the hypothalamo-pituitary axis could occur outside of the hypothalamus and limbic system, in the intergeniculate leaflet of the lateral geniculate body. These signaling pathways together with the demonstration of gender specific SCN input to neuroendocrine cells and local estrogen formation in the extended biological clock during the critical developmental period lead us to conclude that the female-specific emergence of circadian gonadotropin secretion is supported by sexual dimorphisms in the biological clock. Further studies are needed to test this proposition and the functional significance of our results.

Significance to human health

The appropriate entrainment of brain functions to the environment is normally achieved by the circadian clock in all species thus far studied, including humans. Altered activity of the biological clock in the absence of gonadal hormones may be a key trigger in initiating and maintaining discomforting side effects of gonadal failure including post partum depression, premenstrual and perimenopausal mood swings and hot flushes. In light of the fact that interactions between the biological clock and the neuroendocrine hypothalamus seem to share similarities in rats and higher primates, the results gained in the current thesis could establish a fundamental hormone-dependent mechanism by which the biological clock functions. This, in turn, may offer new avenues to enhance the clinical management of the aforementioned symptoms.

PEER REVIEWED PUBLICATIONS DIRECTLY RELEVANT TO THIS DISSERTATION:

1. Horvath TL (1997) Suprachiasmatic efferents avoid phenestrated capillaries but innervate neuroendocrine cells including those producing dopamine. Endocrinology 138:1312-1320. *Impact factor: 4.7*
2. van der Beek EM, Horvath TL, Wiegant VM, van den Hurk R, Buijs RM (1997) Evidence for a direct neuronal pathway from the suprachiasmatic nucleus to the gonadotropin-releasing hormone system: Combined tracing and light- and electron-microscopical immunocytochemical studies. J Comp Neurol 384:569-579. *Impact factor: 3.8*
3. Horvath TL (1998) An alternate pathway for visual signal integration into the hypothalamo-pituitary axis: retinorecipient intergeniculate neurons project to various regions of the hypothalamus and innervate neuroendocrine cells including those producing dopamine. Journal of Neuroscience 18:1546-1558. *Impact factor: 7.9*
4. Horvath TL, Cela V, van der Beek EM (1998) Gender specific apposition of vasoactive intestinal peptide-containing axons on gonadotrophin-releasing hormone neurons in the rat. Brain Research 795:277-281. *Impact factor: 2.5*
5. Horvath TL, Wikler KC (1998) Aromatase in developing sensory systems of the rat. J Neuroendocrinology (in press). *Impact factor: 2.9*

ABSTRACTS OF INTERNATIONAL CONFERENCES DIRECTLY RELEVANT TO THIS DISSERTATION:

1. Horvath TL, Leranth C, Naftolin F (1994) Suprachiasmatic (SCN) and Subparaventricular (SPV) efferents target median eminence projective neurons containing calbindin (CB, tyrosine hydroxylase (TH), galanin (GAL) and β -endorphin (β -END). *3rd International Congress of Neuroendocrinology, Budapest, Hungary. Neuroendocrinology 60 (Suppl. 1):11.*

2. **Horvath T, Jakab R, Roa L, Harada N, Garcia-Segura LM, Naftolin F** (1994) The role of estrogen/aromatase in brain differentiation and development. *10th Biennial Meeting of the International Society for Developmental Neuroscience*, San Diego, CA, abstract 92, p: 68.
3. **Horvath TL, Roa-Pena L, Wikler K, Naftolin F** (1994) Estrogen synthetase (aromatase) immunoreactivity in the developing rat visual system. *24th Ann Meeting of Soc for Neurosci*, Miami, FL, Abstract 364.7, p: 869.
4. **Roa-Pena L, Naftolin F, Horvath TL** (1994) Presence of estrogen synthetase (aromatase) in the developing rat olfactory system. *24th Ann Meeting of Soc for Neurosci*, Miami, FL, Abstract 364.5, p: 869.
5. **Horvath TL, Roa-Pena L, Naftolin F** (1996) Aromatase in the developing sensory system of the rat. *Fourth International Aromatase Conference*, Tahoe City, CA, S-39, p:19.
6. **Horvath TL** (1996) Evidence that circadian and visual signals from the SCN and the LGN are integrated on neuroendocrine dopamine cells in the rat hypothalamus. *26th Annual Meeting of the Society for Neuroscience*, Washington, DC, Abstract #:65.6, p:150.
7. **Horvath TL, Shughurue PJ, Merchenthaler I** (1998) Evidence for local formation and action of gonadal steroids in the developing and adult rat intergeniculate leaflet. *6th Meeting of the Society for Research on Biological Rhythms*, Amelia Island Plantation, Jacksonville, FL, Abst#244, p:173.

OTHER PUBLICATIONS:

Original contributions (published or in press):

1. **Horvath TL, Leranth C, Naftolin F** (1992) Presence of calbindin and lack of parvalbumin in progesterone receptor-containing neurons of the monkey mediobasal hypothalamus. *Neuroscience* 50:309-314. *Impact factor: 3.9*
2. **Horvath TL, Naftolin F, Leranth C** (1992) β -endorphin innervation of dopamine neurons in the rat hypothalamus; a light and electron microscopic double immunostaining study. *Endocrinology* 131:1547-1555. *Impact factor: 4.7*
3. **Horvath TL, Naftolin F, Leranth C** (1992) GABAergic and catecholaminergic innervation of mediobasal hypothalamic β -

- endorphin cells projecting to the medial preoptic area. *Neuroscience* 51:391-399. *Impact factor*: 3.9
4. Horvath TL, Naftolin F, Kalra SP, Leranth C (1992) Neuropeptide Y innervation of β -endorphin-containing cells in the rat mediobasal hypothalamus. A light and electron microscopic double-immunostaining study. *Endocrinology* 131: 2461-2467. *Impact factor*: 4.7
 5. Horvath TL, Naftolin F, Leranth C (1993) Luteinizing hormone-releasing hormone and gamma aminobutyric acid neurons in the medial preoptic area are synaptic targets of dopamine axons originating in anterior periventricular areas. *J Neuroendocrinology* 5:71-79. *Impact factor*: 2.9
 6. Horvath TL, Shanabrough M, Naftolin F, Leranth C (1993) Neuropeptide Y innervation of estrogen-induced progesterone receptor-containing dopamine cells in the monkey hypothalamus: A triple labeling light and electron microscopic study. *Endocrinology* 133:405-414. *Impact factor*: 4.7
 7. Jakab RL, Horvath TL, Leranth C, Harada N, Naftolin F (1993) Aromatase immunoreactivity in the brain: sex hormone-sensitive hypothalamic neurons and an unresponsive "limbic ring" of the lateral septum-bed nucleus-amygdala complex. *J Steroid Biochemistry and Molecular Biology* 44:481-498. *Impact factor*: 1.5
 8. Xu B, Sahu A, Crowley WR, Leranth C, Horvath T, Kalra SP (1993) Role of neuropeptide Y in episodic luteinizing hormone release in ovariectomized rats: an excitatory component and opioid involvement. *Endocrinology* 133: 747-754. *Impact factor*: 4.7
 9. Dube MG, Horvath TL, Leranth C, Kalra PS, Kalra SP (1994) Naloxone suppresses the feeding evoked by intracerebroventricular galanin injection. *Physiology and Behavior* 59:811-813. *Impact factor*:
 10. Herbison AE, Horvath TL, Naftolin F, Leranth C (1995) Distribution of estrogen receptor-immunoreactive cells in the monkey hypothalamus: Relationship to neurons containing luteinizing hormone-releasing hormone and tyrosine hydroxylase. *Neuroendocrinology* 61:1-10. *Impact factor*: 2.4

11. Horvath TL, Kalra SP, Leranath C, Naftolin F (1995) Galanin neurons exhibit estrogen receptor immunoreactivity in the rat mediobasal hypothalamus. *Brain Research* 675:321-324. *Impact factor: 2.5*
12. Horvath TL, Kalra SP, Naftolin F, Leranath C (1995) Morphological evidence for a galanin -opiate interaction in the rat mediobasal hypothalamus. *J Neuroendocrinology* 7:579-588. *Impact factor: 2.9*
13. Naftolin F, Horvath TL, Jakab RL, Leranath C, Harada N, Balthazart J (1996) Aromatase immunoreactivity in axon terminals of the vertebrate brain; an immunocytochemical study on quail, rat, monkey and human tissues. *Neuroendocrinology* 63: 149-155. *Impact factor: 2.4*
14. Horvath TL, Naftolin F, Leranath C, Sahu A, Kalra SP (1996) Morphological and pharmacological evidence for neuropeptideY-galanin interaction in the rat hypothalamus. *Endocrinology* 137:3069-3077. *Impact factor: 4.7*
15. Naftolin F, Mor G, Horvath TL, Luquin S, Fajer AB, Kohen F, Garcia-Segura LM (1996) Synaptic remodeling in the arcuate nucleus during the estrus cycle is induced by estrogen and precedes the midcycle gonadotrophin surge. *Endocrinology* 137:5576-5580. *Impact factor: 4.7*
16. Diano S, Naftolin F, Horvath TL (1997) Gonadal steroid target AMPA glutamate receptor-containing neurons in the rat hypothalamus, septum and amygdala: A morphological and biochemical study. *Endocrinology* 138:778-789. *Impact factor: 4.7*
17. Horvath TL, Garcia-Segura LM, Naftolin F (1997) Lack of gonadotrophin positive feedback in the male rat predicts lack of estrogen-induced synaptic plasticity in the arcuate nucleus. *Neuroendocrinology* 65:136-141. *Impact factor: 2.4*
18. Pu S, Horvath TL, Diano S, Naftolin F, Kalra PS, Kalra SP (1997) Evidence showing that β -endorphin regulates cyclic guanosine 3',5'-monophosphate (cGMP) efflux: Anatomical and functional support for an interaction between opiates and nitric oxide. *Endocrinology* 138: 1537-1543. *Impact factor: 4.7*
19. Horvath TL, Bechmann I, Kalra SP, Naftolin F, Leranath C (1997) Heterogeneity in the neuropeptide Y-containing neurons of the rat arcuate nucleus: GABAergic and non-GABAergic subpopulations. *Brain Research* 756: 283-286. *Impact factor: 2.5*

20. Horvath TL, Roa-Pena L, Jakab RL, Simpson E, Naftolin F (1997) Aromatase in axonal processes of early postnatal hypothalamic and limbic areas including the cingulate cortex. *J Steroid Biochem and Molecular Biology* 61:349-357. *Impact factor: 1.5*
21. Kluge A, Hailer NP, Horvath TL, Bechmann I, Nitsch R (1998) Tracing of the entorhinal-hippocampal pathway in vitro. *Hippocampus* 8:57-68. *Impact factor: 3.5*
22. Diano S, Naftolin F, Horvath TL (1998) Kainate glutamate receptors (GluR5-7) in the rat arcuate nucleus; Their relationship to tanycytes, astrocytes, neurons and gonadal steroid receptors. *J Neuroendocrinology* 10(4):239-247. *Impact factor: 2.9*
23. Diano S, Naftolin F, Goglia F, Horvath TL (1998) Fasting-induced increase in type II iodothyronine deiodinase activity and messenger ribonucleic acid levels is not reversed by thyroxine in the rat hypothalamus. *Endocrinology* 139:2879-2884. *Impact factor: 4.7*
24. Diano S, Kalra SP, Horvath TL (1998) Leptin receptor immunoreactivity are associated with the Golgi apparatus of hypothalamic cells. *J Neuroendocrinology* 10 (9):647-650. *Impact factor: 2.9*
25. Diano S, Naftolin F, Goglia F, Horvath TL (1998) Segregation of intra- and extrahypothalamic neuropeptide Y and catecholaminergic inputs on paraventricular neurons including those producing TRH. *Regulatory Peptides (in press)*. *Impact factor: 1.1*
36. Diano S, Naftolin F, Goglia F, Csernus V, Horvath TL (1998) Evidence for a monosynaptic pathway between the arcuate nucleus expressing glial type II iodothyronine 5' deiodinase mRNA and the median eminence-projective TRH cells of the rat paraventricular nucleus. *J Neuroendocrinology (in press)*. *Impact factor: 2.9*
27. Diano S, Kalra SP, Sakamoto H, Horvath TL (1998) Leptin in estrogen receptor-containing neurons of the female rat hypothalamus. *Brain Research (in press)*. *Impact factor: 2.5*

Reviews/Book chapters

1. Leranath C, Shanabrough M, Naftolin F, Horvath TL (1994) Electron microscopic double and triple labeling immunocytochemistry in the elucidation of synaptological interaction between ovarian steroid-

- sensitive neurons and circuits. In: *Methods in Neuroscience*, Conn MP(ed): Neurobiology of steroids, de Kloet ER, Sutanto W (volume eds), pp: 403-435.
2. Horvath TL, Leedom L, Garcia-Segura LM, Naftolin F (1995) Estrogen-induced hypothalamic synaptic plasticity; Implications for the regulation of gonadotrophins. *Current Opinion in Endocrinology and Diabetes*, M.S. Smith (ed.), Vol. 2:186-190.
 3. Leranath C, Naftolin F, Shanabrough M, Horvath TL (1995) Neuronal circuits regulating gonadotropin release. In: *The neurobiology of puberty* (Plant TM and Lee PA eds.), *J. of Endocrinology Limited*, pp:55-73.
 4. Naftolin F, Leranath C, Horvath TL, Garcia-Segura LM (1995) Potential neuronal mechanisms of estrogen actions in synaptogenesis and synaptic plasticity. *Cellular and Molecular Neurobiology* 16(2):213-223.
 5. Kalra SP, Horvath T, Naftolin F, Xu B, Pu S, Kalra PS (1997) The language of the hypothalamus for the GnRH system. *J. Neuroendocrinology* 9:569-576.
 6. Horvath TL, Garcia-Segura LM, Naftolin F (1997) Control of gonadotrophin feedback: The role of estrogen-induced hypothalamic synaptic plasticity. *Gynec. Endocrin.* 11:139-143.
 7. Kalra, SP, Xu B, Dube MG, Pu S, Horvath TL, Kalra PS (1998) Interacting appetite regulating pathways in the hypothalamic regulation of body weight. *Endocrine Reviews. Impact factor: 22.2*
 8. Kalra SP, Horvath TL (1998) Neuroendocrine interactions between galanin, opioids, and neuropeptide Y in the control of reproduction and appetite. *Annals of the New York Academy of Sciences*.

Miscellaneous

1. Naftolin F, Horvath TL (1994) Providing a "Noah's Ark" for research in the reproductive sciences by fostering young investigators: A role for the SGI (editorial). *Journal of the Society for Gynecologic Investigations* 1(4):246.

Published abstracts of international conferences

1. Horvath TL, Naftolin F, Leranath C (1991) Luteinizing hormone-releasing hormone (LHRH) and GABA neurons in the medial

preoptic area (MPO) of female rats are synaptic targets of dopamine axon terminals originating in the sexually dimorphic anteroventral periventricular nucleus (AVPN) and not in the zona incerta (ZI). *Third IBRO World Congress of Neuroscience*, Montreal, Canada. p:275.

2. Horvath TL, Naftolin F, Leranth C (1991) Mediobasal hypothalamic (MBH) β -endorphin neurons projecting to the medial preoptic area (MPO) are synaptic targets of catecholamine fibers. *Third IBRO World Congress of Neuroscience*, Montreal, Canada. p:275.
3. Horvath TL, Naftolin F, Leranth (1991) Presence of calbindin (CB) and lack of parvalbumin (PV) in progesterone receptor-containing (PR) neurons of the primate hypothalamus. *21st Annual Meeting of Soc for Neurosci.*, New Orleans, LU, Abstract, 17:1230.
4. Horvath TL, Kalra SP, Naftolin F, Leranth C (1993) Evidence for a neuropeptide Y-Galanin- β -endorphin pathway in the rat hypothalamus. *23rd Annual Meeting of Soc for Neurosci.*, Washington, DC, abstract: 696.1
5. Horvath TL, Roa-Pena L, Blumenfield Z, Naftolin F (1995) Aromatase immunoreactive neurons and neural processes in the hypothalamus of human fetuses and adult subjects. *42nd Ann Meeting of the Society for Gynecologic Investigation*, Chicago, IL. abstract# O54, J. Soc. Gyn. Invest. 2:73.
6. Horvath TL, Roa-Pena L, Pahuja S, Hochberg R, Naftolin F (1995) Aromatase in the hindbrain of developing rats. *Fourth IBRO World Congress of Neuroscience*, Kyoto, Japan, Abstract#: C2.46, p:241.
7. Sahu A, Xu B, Horvath T, Leranth C, Crowley WR, Kalra SP (1995) Evidence that galanin (GAL) may participate in stimulation of LH secretion by neuropeptide Y (NPY). *25th Annual Meeting of the Society for Neuroscience*, San Diego CA, Abstract #:112.2, p:263.
8. Diano S, Naftolin F, Leranth C, Horvath TL (1995) Ionotropic glutamate receptors in the rat hypothalamus and limbic system: Coexistence with gonadal steroid receptors. *25th Annual Meeting of the Society for Neuroscience*, San Diego CA, Abstract #:176.14, p:430.
9. Naftolin F, Diano S, Lerma J, Garcia-Segura LM, Leranth C, Horvath TL (1995) Kainate glutamate receptors in tanycytes and type II

- astrocytes of the rat arcuate nucleus. *25th Annual Meeting of the Society for Neuroscience*, San Diego CA, Abstract #: 742.2, p:1888.
10. Horvath TL, Naftolin F (1995) Bilateral enucleation at birth diminishes simulated midcycle gonadotropin surges in adult female rats. *25th Annual Meeting of the Society for Neuroscience*, San Diego CA, Abstract #: 743.13, p:1893.
 11. Horvath TL, Naftolin F (1996) Estradiol treatment increases the number of synapses targeting β -endorphin cells. *43rd Ann Meeting of the Society for Gynecologic Investigation*, Philadelphia, PA. abstract# 208, *Journal of the Society for Gynecologic Investigation*, 3:162A.
 12. Diano S, Naftolin F, Horvath TL (1996) Testosterone increases AMPA GluR 2/3 protein- and GluR 2 mRNA levels in the CA1 region of the hippocampus and decreases neuronal damage during ischemia. *26th Annual Meeting of the Society for Neuroscience*, Washington, DC, Abstract #:41.11, p: 82.
 13. Scheidereiter A, Hailer NP, Bechmann I, Horvath TL, Nitsch R (1997) In vitro monitoring of the perforant path in organotypic entorhinal-hippocampal complex slice cultures. *27th Annual Meeting of the Society for Neuroscience*, New Orleans, LA, Abstract # 865.4, p:2224.
 14. Horvath TL, Keefe DL, Leranath C (1997) A retino-recipient suprachiasmatic area of the monkey (*Cercopithecus aethiops*) hypothalamus is directly connected to hypothalamic LHRH neurons. *27th Annual Meeting of the Society for Neuroscience*, New Orleans, LA, Abstract #16.7, p:21.
 15. Diano S, Naftolin F, Goglia F, Csemus V, Horvath TL (1997) A signaling pathway between the arcuate nucleus and the neuroendocrine thyrotropic (TRH) neurons in female rats. *27th Annual Meeting of the Society for Neuroscience*, New Orleans, LA, Abstract # 63.10, p:141.
 16. Naftolin F, Park M, Kagiya A, Takahasi K, Brindle M, Horvath TL (1997) Decreased afferent connectivity of GnRH neurons in surgically menopausal monkeys (*Maccaca Fascicularis*) following chronic hormonal replacement therapy with premarin or premarin plus provera. *27th Annual Meeting of the Society for Neuroscience*, New Orleans, LA, Abstract #699.8, p:1796.

17. Sison AV, Horvath TL, Chowen JA, Garcia-Segura LM and Naftolin F (1997) A potential influence of ovarian cycle day on the presence of polysialic acid neural cell adhesion molecule (PSA-NCAM) in the rat hypothalamus. *27th Annual Meeting of the Society for Neuroscience*, New Orleans, LA, Abstract #770.14, p: .
18. Pu S, Jain M, Horvath TL, Diano S, Kalra SP (1997) Coexistence of Neuropeptide y (NPY) and γ -amino butyric acid (GABA) in the hypothalamus: Interactive effects on feeding. *27th Annual Meeting of the Society for Neuroscience*, New Orleans, LA, Abstract #528.3, p:1344.
19. Lephart ED, Watson MA, Mathias L, Rhees RW, Diano S, Horvath TL (1997) Co-localization of aromatase cytochrome P450 and calbindin-D28K and androgen regulation of calbindin-D28K during perinatal development. *27th Annual Meeting of the Society for Neuroscience*, New Orleans, LA, Abstract #139.10, p:342.
20. Diano S, Goglia F, Naftolin F, Horvath TL (1997) GABAergic NPY input of parvocellular TRH cells: its origin in the arcuate nucleus and regulation by thyroid hormones. *4th International NPY Conference*, London, UK Abstract#:OC34, Regulatory Peptides 71:217.
21. Horvath T, Park MC, Garcia-Segura LM, Naftolin F (1998) Hormonal control of brain cells: opposite effects of estrogen versus estrogen-progestin replacement on GnRH cell membrane composition in female monkey brains. *45th Annual Meeting of the Society for Gynecologic Investigation*, Atlanta, GA, Abstr#T227; *Journal of S.G.I.*, 5(Suppl. 2): 227.
22. Bechmann I, Diano-Horvath S, Naftolin F, Leranth C, Nitsch R, Horvath TL (1998) Estrogen induces parvalbumin expression and increased AMPA receptor content in endorphin cells: Implications for glutamate involvement in hypothalamic neurotoxicity. *1998 Forum of European Neuroscience*, Berlin, Germany; *European Journal of Neuroscience* P:114; Abst#51.01.
23. Diano S, van den Pol AN, Horvath TL (1998) Hypocretin (orexin)-containing neuronal network in the primate hypothalamus and its relationship to fasting-induced c-Fos expressing cells. *28th Annual Meeting of the Society for Neuroscience*, Los Angeles, CA, Abstract #11.6, p:12.

24. Horvath TL, Diano S van den Pol AN (1998)-Hypocretin (orexin)-containing neurons make synaptic contact with arcuate nucleus NPY- POMC-producing cells that express leptin receptors in rodent and primate; novel hypothalamic circuit involved in energy homeostasis. *28th Annual Meeting of the Society for Neuroscience*, Los Angeles, CA, Abstract #11.7, p:12.