MOLECULAR BASIS *of* AGING

Edited by
Alvaro Macieira-Coelho, M.D., D.Sc., D.h.c.
Research Director
INSERM
Versailles, France

CRC Press

Boca Raton New York London Tokyo

Library of Congress Cataloging-in-Publication Data

Molecular basis of aging/edited by Alvaro Macieira-Coelho.

p. cm.

Includes bibliographical references and index.

ISBN 0-8493-4786-6

1. Aging--Molecular aspects. 2. Aging--Genetic aspects.

I. Macieira-Coelho, Alvaro, 1932-

[DNLM: 1. Aging--genetics. 2. Mammals--genetics. 3. Evolution.

WT 104 M7174 1995]

QP863.M683 1995

599'.0372--dc20

DNLM/DLC

for Library of Congress

95-15170

CIP

This book contains information obtained from authentic and highly regarded sources. Reprinted material is quoted with permission, and sources are indicated. A wide variety of references are listed. Reasonable efforts have been made to publish reliable data and information, but the author and the publisher cannot assume responsibility for the validity of all materials or for the consequences of their use.

Neither this book nor any part may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, microfilming, and recording, or by any information storage or retrieval system, without prior permission in writing from the publisher.

All rights reserved. Authorization to photocopy items for internal or personal use, or the personal or internal use of specific clients, may be granted by CRC Press, Inc., provided that \$.50 per page photocopied is paid directly to Copyright Clearance Center, 27 Congress Street, Salem, MA 01970 USA. The fee code for users of the Transactional Reporting Service is ISBN 0-8493-4786-6/95/\$0.00+\$.50. The fee is subject to change without notice. For organizations that have been granted a photocopy license by the CCC, a separate system of payment has been arranged.

CRC Press, Inc.'s consent does not extend to copying for general distribution, for promotion, for creating new works, or for resale. Specific permission must be obtained in writing from CRC Press for such copying.

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida 33431.

© 1995 by CRC Press, Inc.

No claim to original U.S. Government works

International Standard Book Number 0-8493-4786-6

Library of Congress Card Number 95-15170

Printed in the United States of America 1 2 3 4 5 6 7 8 9 0

Printed on acid-free paper

AGE-RELATED CHANGES IN
NEUROPEPTIDERGIC NEURONS
IN THE HUMAN HYPOTHALAMUS

Jiang-Ning Zhou and Dick F. Swaab

CONTENTS

I. Introduction 528

II. Suprachiasmatic Nucleus 529

III. Sexually Dimorphic Nucleus (Intermediate Nucleus,
INAH-1) 533

IV. Paraventricular and Supraoptic Nucleus 533

 A. Vasopressin and Oxytocin 533

 B. Corticotropin-Releasing Hormone 536

 C. Thyrotropin-Releasing Hormone (TRH) 538

 D. Other Peptides 540

V. Infundibular (Arcuate) Nucleus 540

 A. Luteinizing Hormone-Releasing Hormone (LHRH) 540

 B. Growth Hormone-Releasing Hormone (GHRH)
 and Somatostatin 542

VI. Lateral Tuberal Nucleus 543

VII. Conclusion	543
Acknowledgments	544
References	544

I. INTRODUCTION

Since 72-year-old Brown-Séquard, more than one century ago, injected himself with testicular extracts and claimed remarkable physical and mental signs of rejuvenation,¹ a close relationship between endocrine changes and aging has been supposed.² Because of its central role in endocrine regulation, the hypothalamus has received the constant attention of gerontologists.³⁻⁵ Over the past decades there has been remarkable progress in the knowledge of hypothalamic neuropeptidergic systems in relation to aging, to which several technical developments have contributed. In the first place, the isolation and characterization of hypothalamic peptides⁶⁻⁸ have made subsequent immunocytochemical localization of neuropeptides possible in the human hypothalamus.⁹ It is now generally accepted that neuropeptides may either be released into the circulation as hormones, or act as neurotransmitters or neuromodulators when released from synapses, and may then influence central processes.¹⁰ In addition, many, if not all, hypothalamic peptides are colocalized with classical transmitters, e.g., acetylcholine, gamma-aminobutyric acid (GABA), or amines such as dopamine.¹¹ Since the classical neurotransmitters show clear changes with aging,¹²⁻¹⁴ changes in the colocalizing neuropeptides may also be expected. Another reason to stress the importance of the hypothalamus for the aging process is that some hypothalamic systems themselves have been causally implicated in aging of the brain^{15,16} and in the life span.¹⁷ The present chapter tries to relate functional changes in aging with alterations in neuropeptidergic neurons of the human hypothalamus.

The hypothalamus is a complex, heterogeneous structure containing a number of nuclei which are often characterized by specific neuropeptides.⁹ Basically, there are two types of hypothalamic peptidergic neurons. The first one is the neuroendocrine type, which releases its peptide as a hormone into the general circulation in the neurohypophysis, e.g., vasopressin (AVP) and oxytocin (OXT), or into the portal circulation of the pituitary, e.g., AVP, luteinizing hormone-releasing hormone (LHRH), thyrotropin-releasing hormone (TRH), and corticotropin-releasing hormone (CRH). The second type of hypothalamic peptidergic neuron is the one that sends its axon into the brain¹⁸ where it terminates synaptically on

other neurons, acting as a neurotransmitter or neuromodulator.¹⁰ The same peptide may be present in both types of neurons, e.g., AVP is present in the hypothalamo-neurohypophysial-system (HNS) as a neurohormone and also in the suprachiasmatic nucleus as a neurotransmitter or neuromodulator.

II. SUPRACHIASMATIC NUCLEUS

The suprachiasmatic nucleus (SCN) is the major circadian pacemaker of the mammalian brain and coordinates hormonal and behavioral circadian rhythms.¹⁹ A relatively large number of neuropeptides have been identified in the human SCN. Neurons that are immunoreactive for vasopressin (AVP), vasoactive intestinal polypeptide (VIP), neuropeptide-Y (NPY), neurotensin (NT), and somatostatin (SOM) are present in the SCN in a characteristic anatomical orientation (Figure 1).²⁰⁻²³ Typical for the human SCN, as compared to monkeys and other animals, are the very large populations of NT and NPY neurons.²³ Colocalization of different neuropeptides in the SCN has been found mainly in rat but also in human.^{24,25} For example, VIP-producing neurons in the SCN were shown to colocalize galanin, AVP,²⁵ gastrin releasing peptide (GRP),²⁶ or GABA.²⁷

Recent observations have revealed marked changes in the volume of AVP subpopulation and AVP cell number of the human SCN in relation to the season.^{28,29} In addition, a day-night fluctuation in the number of AVP expressing neurons in the SCN was found to be present, but only in young subjects,³⁰ which suggests a diminution of circadian fluctuations of the SCN in human aging. Age-related changes in circadian rhythms have indeed been reported in man as well as in other species.³¹⁻³³ Furthermore, a decrease in the number of AVP cells and total cell number was found in subjects aged 80 to 100, while these changes were even more pronounced in Alzheimer's disease patients than in controls (Figure 2).²¹

A different pattern from that of the AVP neurons was found recently in the number of VIP expressing neurons of the human SCN during aging (Figure 3).^{34,35} The VIP cell number in the female SCN remained very stable during the life span. In males, however, the number of VIP neurons in the SCN reached its peak value in young males (10 to 40 years of age). Subsequently, a dramatic decrease in the number of VIP neurons in SCN was found in the middle-aged subjects (41 to 65 years of age). A significant reduction in the number of VIP expressing neurons was found in the old-age group (65 to 92 years of age). An age-dependent sex difference was observed in the SCN: males of 10 to 40 years of age had twice as many VIP neurons in the SCN as females. Due to the age-related fluctuations in VIP cell number in males, this sex difference was reversed in the middle-aged

Figure 1

Diagram showing the organization of the human suprachiasmatic nucleus (SCN). The distribution of vasopressin (VP), vasoactive intestinal polypeptide (VIP), neurotensin (NT), and neuropeptide-Y (NPY) neurons and fibers is shown at three levels, from rostral to caudal. (From Moore, R. Y., *Progress in Brain Research*, Swaab, D. F., Hofman, M. A., Mirmiran, M., Ravid, R., and Van Leeuwen, F. W., Eds., Elsevier, Amsterdam, 1992. With permission.)

group, the females having twice as many VIP neurons in the SCN. After 65 years, sex differences were no longer found (Figure 3).^{34,35} Since the SCN is the clock of the brain, the morphological sex differences may be related to sex differences that have been found in circadian control mechanisms of hamsters³⁶ and humans.³⁷ In addition, it has been found in rat that VIP expressing neurons from the SCN directly innervate LHRH neurons that are involved in reproductive functions.³⁸ Although such a connection

Figure 2

The number of vasopressin (AVP) immunoreactive neurons in the human SCN. The number of AVP neurons in the 81- to 100-year-old group decreased as compared to the 61- to 80-year-old subjects ($p < 0.02$). Data are represented as mean $\pm$ SEM. (From Swaab, P. F., Roozendaal, B., Ravid, R., Velis, D. N., Gooren, L., and Williams, R. S., *Progress in Brain Research*, Vol. 72, De Kloet, R. et al., Eds., Elsevier, Amsterdam, 1987. With permission.)

should be confirmed in the human hypothalamus, the sex difference in the number of VIP neurons in the SCN and the difference in the AVP subnucleus of the SCN according to sexual orientation also suggests a possible role of the biological clock in reproduction or sexual behavior.^{39,40} Females showed a very stable number of VIP neurons in the SCN with aging, whereas a decrease in the number of VIP neurons in the SCN was found in middle-aged males (Figure 3).³⁵ It is tempting to relate the changes in the peptidergic neurons of the SCN to functional changes, e.g., in circadian rhythmicity.

Although the number of AVP neurons in the SCN in 60- to 80-year-old subjects did not differ from the number found in young subjects,⁴¹ the circadian fluctuation in the number of AVP neurons in the human SCN diminished in subjects older than 50 years.³⁰ The number of AVP neurons

Figure 3

Lifespan changes in the number of vasoactive intestinal polypeptide (VIP) immunoreactive neurons in the human SCN. The blank bar indicates the males, the hatched bar indicates the females. The SCN of young males (10 to 40 years) contains twice as many neurons as that of young females ($p < 0.02$). This sex difference reverses in middle-aged subjects ($p < 0.02$). Since the number of VIP neurons decreased after 40 years, the cell number is significantly lower in elderly males than in young males ($p < 0.05$). (From Zhou, J. N., Hofman, M. A., and Swaab, D. F., *Neurobiol. Aging*, in press. With permission.)

did not decrease until after 80 years of age,²¹ suggesting that the circadian fluctuations in AVP neurons disappear earlier in the process of aging than the number of neurons expressing AVP.

The relation between VIP and changed circadian fluctuations is less clear. Circadian changes were found in plasma VIP levels in elderly males (74 to 85 years) and in subjects that were 10 years younger (65 to 75 years).^{42,43} However, these blood levels of VIP probably do not come from SCN neurons. The stability of VIP neurons, especially in the female SCN, might be related to some stable rhythms in normal human aging, such as the rhythmicity of temperature, prolactin, or cortisol.⁴⁴⁻⁴⁷ On the other hand, it has been proposed that the decreased AVP cell number might be the basis for circadian disturbances in aging and Alzheimer's disease.²¹ Some observations suggest that a certain degree of plasticity remains present in the SCN neurons in aging.³² SCN neurons in old rats seem to continue to respond to increased input, since increased amounts of light improved and restored circadian rhythms in these animals.⁴⁸ Such a nonpharmacological treatment of disturbances of the sleep-wake pattern may be of clinical importance, not only in aging but also in Alzheimer's disease, where it may reduce nightly restlessness.³²

Other changes in peptidergic SCN neurons may also be present in aging. A dense plexus of substance P (SP) immunoreactive axons that overlaps the distribution of VIP expressing perikarya has recently been reported in the human SCN (Moore et al., personal communication, 1994). Interestingly, the number of SP neurons in the SCN of aged hamsters was increased three to four times compared with adult animals, day and night.⁴⁹ However, observations on this peptidergic neuron population during the process of aging have not yet been performed in humans.

III. SEXUALLY DIMORPHIC NUCLEUS (INTERMEDIATE NUCLEUS, INAH-1)

The sexually dimorphic nucleus of the preoptic area (SDN) is identical to the intermediate nucleus as mentioned by Braak and Braak,⁵⁰ and to the INAH-1 described by Allen et al.⁵¹ In girls, the SDN shows a decreasing cell number during prepubertal development, leading to sexual dimorphism.⁵² The SDN in the young adult human brain is twice as large in males as in females and contains twice as many cells.⁵³ During aging, a decrease in cell number is found in both sexes. In males the cell number decreases strongly after 50 years of age, in females a second phase of cell loss appears after the age of 70 (Figure 4).^{52,54} The sharp decline in cell numbers in the SDN later in life might be related to the hormonal changes which accompany both male and female senescence⁵⁴ and to the decrease in male sexual activity,⁵⁵ although the causality of such a relationship has not yet been established. A good marker for the neuronal content of the majority of SDN neurons is not available at present. Only a few TRH neurons have so far been identified in this nucleus.⁵⁶ Such TRH-positive neurons have also been identified in the rat.⁵⁷

IV. PARAVENTRICULAR AND SUPRAOPTIC NUCLEUS

The paraventricular (PVN) and supraoptic (SON) nucleus and their axons form the hypothalamo-neurohypophyseal-system (HNS). Immunocytochemical studies have established that in addition to oxytocin (OXT) and vasopressin (AVP), a large number of other neuropeptides are synthesized by neurons of the PVN and SON.

A. Vasopressin and Oxytocin

AVP and OXT are produced by the magnocellular neurosecretory system of the hypothalamic SON and PVN projecting to the neurohypo-

Figure 4

Age-related changes in the total cell number of the sexually dimorphic nucleus of the human hypothalamus. The general trend in the data is enhanced by using smoothed growth curves. Note that in males the SDN cell number steeply declines between the ages of 50 and 60 years, whereas in females a more gradual cell loss is observed around the age of 80 years. These curves demonstrate that the reduction in cell number in the human SDN in senescence is a nonlinear, sex-dependent process. (From Hofman, M. A. and Swaab, D. F., *J. Anat.*, 164, 55, 1989. With permission.)

physis where they are released into the circulation. AVP acts as an antidiuretic hormone on the kidney⁵⁸ and as a vasopressor on the blood vessels.⁵⁹ OXT is involved in labor and lactation⁶⁰ and in other reproductive processes.⁹ In addition to these functions, magnocellular OXT- and AVP-containing cells in the SON and PVN coexpress tyrosine hydroxylase, suggesting the possibility of dopamine production.^{61,62} AVP neurons of the PVN project into the brain,¹⁸ and animal experiments suggest that they may influence central processes such as certain aspects of memory.^{63,64} Peptides have been given as substitution therapy in disorders where a deficiency in these systems was presumed. However, clinical trials in which AVP or its analogs were administered to patients with Alzheimer's disease (AD) yielded inconsistent results.⁶⁵ OXT also has central effects, e.g., on the inhibition of food intake and on maternal and reproductive behavior.^{66,67} In males, OXT might be involved in sexual arousal and ejaculation.⁶⁸ In rodents, depending on the strain, either activation or deterioration of the HNS during aging has been reported.⁶⁹ In contrast to the rodent data, nearly all human data point to an activation of the HNS with aging, especially of the vasopressinergic neurons. The mean profile area of AVP cells increased after the age of 80 years, suggesting an increased

peptide production from this age onwards.⁷⁰ The OXT cells in these nuclei showed no such change.⁷⁰ Neuronal hypertrophy has also been described in unidentified human SON and PVN neurons in aging.⁷¹ Although the correlation between cell size and peptide production is well established in adulthood, one might wonder whether this relationship would also persist in senescence. Since, for example, lipofuscin is known to accumulate in many types of aging nerve cells, this may theoretically cause an enlargement of cytoplasmic volume. Therefore, nucleolar size, which is a reliable parameter for neurosecretory activity, was also determined in the AVP and OXT cells in the same human material. A significantly increased nucleolar diameter was again found in AVP neurons of the SON and PVN in senescent subjects and not in the OXT cells.⁷²

These findings confirmed the idea that the previously observed increase in cell size was indeed due to enhanced neurosecretory activity. However, it could not be excluded from those data that the observed activation of neurosecretory AVP cells was a compensation for cell loss from the PVN or SON. Total cell number, and OXT and AVP cell numbers, have therefore also been determined in the PVN and SON. The results revealed that no significant differences in volume or total cell number were present in either the PVN or SON between young and old control subjects.⁷³⁻⁷⁵ Furthermore, a gradual increase in the number of AVP expressing neurons was even observed in the human PVN and SON with aging (Figure 5).⁷⁶ These findings are in line with the increased cellular⁷⁰ and nucleolar size⁷² that indicated increased AVP synthesis in this nucleus during aging. To further substantiate the hyperactivity of HNS neurons in aging, the size of the Golgi apparatus (GA) was determined in the human SON and PVN in a recent study. The GA, indeed, showed a clear increase in size and intensity with age in controls and Alzheimer patients (Figure 6).⁷⁷ All these indications for activation of AVP are supported by the presence of a gradual increase in human plasma AVP levels during aging.^{4,78,79} In addition, an increase in AVP secretion upon osmotic stimulation was found in elderly subjects.⁸⁰⁻⁸⁴ Since the activation of AVP neurons in aging is probably due to loss of AVP receptors in the kidney,^{85,86} the observed activation of the HNS in senescence might be considered as a compensatory activation. This mechanism, however, must still be confirmed in humans.

The number of OXT expressing neurons in the PVN was found to remain constant during aging (Figure 7),⁸⁷ which is in line with the absence of morphological signs of activation in these cells in senescence.^{70,72} The remarkable stability of the activated SON and PVN neurons in aging, and also in Alzheimer's disease, supports our concept that activated neurons may be able to withstand the processes of aging or neurodegeneration better — a hypothesis that is paraphrased as "use it or lose it".⁸⁸

Figure 5

Linear regression between AVP cell number in PVN and age. A significant correlation between age and cell number was found in control subjects ($r = 0.583$, $n = 20$; $p < 0.01$). Old control subjects had a significantly higher cell number as compared with young controls. This increase in AVP expressing neuron number with age does not occur in Alzheimer's disease (AD), as indicated by the minimum convex polygon. (From Van der Woude, P. F., Goudsmit, E., Wierda, M., Purba, J. S., Hofman, M. A., Bogte, H., and Swaab, D. F., *Neurobiol. Aging*, in press. With permission.)

B. Corticotropin-Releasing Hormone

Corticotropin-releasing hormone (CRH) immunoreactive neurons are restricted to the PVN in the human hypothalamus.^{89,90} As in rat, the CRH neurons of the human PVN are parvicellular.⁹⁰ However, in the rat the CRH neurons are localized in a strictly defined subnucleus of the PVN,⁹¹⁻⁹³ while they are spread throughout the entire PVN in humans, except for the most rostral part where they are absent.⁹⁰ CRH plays a key role in the stress response of the hypothalamic-pituitary-adrenal (HPA) axis. CRH neurons appeared to be activated in aging as was shown by a number of parameters. In the first place, the number of CRH immunoreactive neurons in the human increases in the PVN (Figure 8).⁹⁴ Another sign of activation of CRH neurons is an increase in the coexpression of AVP as appeared from animal experiments.⁹³ Interestingly, an age-dependent increase in the colocalization of AVP was also found in the parvicellular CRH neurons in the human PVN with aging.⁹⁰ Colocalization was especially noted in older control subjects (aged 43 to 91 years), whereas no colocalization was present in younger subjects (aged 23 to 37 years). In addition, an increase in cortisol with age was found in post-mortem cerebrospinal fluid.⁹⁵

Figure 6

Activation of the Golgi apparatus in supraoptic and paraventricular nucleus (SON) and (PVN) neurons of the human hypothalamus as indicated by an increase in size and staining intensity of MG-160, a structural sialoglycoprotein of the medial cisternae. (A) Supraoptic neuron from a 29-year-old control, and (B) from a 73-year-old control. From Lucassen, P. J., Ravid, R., Gonatas, N. K., and Swaab, D. F., *Brain Res.*, 632, 105, 1993. With permission.)

All these findings are in agreement with hormone measurements in the periphery, indicating an activation of the HPA-axis during aging.^{91,96} Moreover, resistance of cortisol secretion to dexamethasone suppression increases with age in controls.^{97,98} Hyperactivity of the HPA-axis is proposed to have severe long-term consequences, since increased levels of corticosteroids have been hypothesized to lead to irreversible hippocampal damage.¹⁶ The hippocampus normally inhibits CRH neurons⁹⁹ and hippocampal lesions may, therefore, subsequently lead to more hyperactivity of CRH neurons. A positive feedback loop would then develop, leading to more CRH activation and more brain damage. Whether such a mechanism is indeed operative in the human brain in aging and AD remains, however, to be proven. There are also observations which do not agree with an activation of the HPA-axis in aging. Measurements of plasma adrenocorticotropin (ACTH) in 25 young and 28 elderly humans revealed no significant differences.¹⁰⁰ Older people did not show significant change in ACTH response after having undergone major surgery. In addition, rhythms of ACTH and cortisol secretion showed no significant

Figure 7

Linear regression between oxytocin (OXT) cell number in the PVN and age. Data of male and female control patients did not differ and were pooled. No statistically significant correlations were observed in either young or old control subjects. Values of male and female Alzheimer's disease (AD) patients are delineated by the minimum convex polygon and were within the range of the controls. (From Wierda, M., Goudsmit, E., Van der Woude, P. F., Purba, J. S., Hofman, M. A., Bogte, H., and Swaab, D. F., *Neurobiol. Aging*, 12, 511, 1991. With permission.)

change with age in healthy elderly subjects. Using frequent plasma sampling techniques,⁴⁷ older subjects also had a normal circadian cortisol rhythm, although the cycle peaked at an earlier hour. These data indicate that the regulatory capacity of the HPA-axis function, though this function is only slightly elevated under basal conditions in elderly subjects, may be diminished. However, on the basis of the activation of CRH neurons we observed in the PVN,^{90,94} central effects of increased CRH activity should also be considered. Increased activity of CRH neurons that project into the brain may lead to symptoms of depression, since CRH when administered centrally in laboratory animals leads to signs and symptoms that are very similar to the symptoms of major depression.¹⁰¹ In addition, extremely activated CRH neurons were indeed observed in depressed patients.⁹⁴

C. Thyrotropin-Releasing Hormone (TRH)

The distribution of TRH neurons in the human hypothalamus has been studied only recently.⁵⁶ The majority of TRH-containing neurons was

Figure 8
Linear regression between age and absolute CRH cell number in the PVN estimated by the disector method (A) and the unfolding method (B). Filled circles and solid lines indicate control subjects; open circles and dashed lines indicate Alzheimer's disease patients. A significant correlation was found between age and absolute CRH cell number for control subjects with both the disector and unfolding methods (respectively, $\rho = 0.66$, $p = 0.02$; $\rho = 0.62$, $p = 0.03$). (From Raadsheer, F. C., Oorschot, D. F., Verwer, R. W. H., Tilders, F. J. H., and Swaab, D. F., *J. Comp. Neurol.*, 339, 447, 1994. With permission.)

present in the PVN, especially in the dorsocaudal part of this nucleus. It is thought that these neurons project to the median eminence and regulate thyroid stimulating hormone (TSH) release. The TRH neurons in the PVN were mostly parvicellular, but a few magnocellular TRH-positive neurons were observed as well. The SON did not show any TRH immunoreactivity. High densities of TRH-positive fibers were seen, not only in the median eminence but also in other hypothalamic areas, e.g., in the PVN, ventromedial nucleus (VM), and in the perifornical area. The large number of TRH-containing fibers apparently terminating on neurons suggests important physiological functions of this neuropeptide as a neurotransmitter or neuromodulator in the human brain. Hypothalamic TRH levels and TRH secretion are reduced in old (24 to 28 months) rats.^{102,103} Up to now, no data are available concerning age-related TRH changes in the human hypothalamus. Increased basal TSH concentrations were reported in elderly people¹⁰⁴ as well as in aged rats.¹⁰⁵ These changes point to the possibility that hypothalamic TRH may be compensatorily increased in elderly subjects. It seems worthwhile, therefore, to study age-related changes of TRH in the human hypothalamus.

D. Other Peptides

The human PVN and SON contain a number of other peptidergic neurons such as prosomatostatin¹⁰⁶ and LHRH.¹⁰⁷ Recently, positive VIP neurons were found to be present in the PVN and SON.^{34,35} In humans, the VIP neurons are spread all over the PVN and are not localized in a particular subnucleus as was found in the rat.¹⁰⁸ In addition, colocalization of galanin has been found in the human SON and PVN, mainly with AVP and less with OXT.²⁵ This means that changes of galanin in the HNS can also be expected in aging.

V. INFUNDIBULAR (ARCUATE) NUCLEUS

The horseshoe-shaped infundibular (or arcuate) nucleus⁵⁰ contains a large number of neurotransmitters and neuropeptides, e.g., catecholamine-containing neurons,⁶¹ somatostatin, neuropeptide Y, and neurotensin. Various neuroendocrine cell types that are involved in the hypothalamo-pituitary-gonadal (HPG) axis are located in this nucleus.

A. Luteinizing Hormone-Releasing Hormone (LHRH)

LHRH is less widely distributed in the brain than many other neuropeptides. LHRH-containing cell bodies are located predominantly in the

Figure 9

Representative photomicrographs of cresyl violet-stained sections of the infundibular nucleus of premenopausal (A) and postmenopausal (B) women. The hypertrophied neurons are distinguished not only by increased soma size, but also by large nuclei, nucleoli, and increased Nissl substance. Bar is 20 μ m for both photomicrographs. (From Rance, N. E., *Progress in Brain Research*, Swaab, D. F., Hofman, M. A., Mirmiran, M., Ravid, R., and Van Leeuwen, F. W., Eds., Elsevier, Amsterdam, 1992. With permission.)

infundibular nucleus and preoptic area.^{109,107,110} LHRH-immunoreactive fibers project to the median eminence, septum, stria terminalis, ventral pallidum, dorsomedial thalamus, olfactory stria, and the anterior olfactory area of the human brain.¹¹¹ Recently, a much wider distribution and greater number of LHRH neurons was found in the human hypothalamus using *in situ* hybridization.¹¹² In this study, three subtypes of LHRH neurons were found as well as two types of small-sized neurons located primarily in the hypothalamus, suggesting that different functional subgroups of LHRH neurons exist in the human brain.¹¹² The colocalization of delta sleep-inducing peptide (DSIP) and LHRH, as found in the arcuate nucleus of aged human subjects, suggests that DSIP may also play a physiological role in LHRH neurons.¹¹³

The menopause represents a dramatic alteration in the function of the HPG axis in women. As a consequence of decreased levels of sex hormones, neuronal hypertrophy has been reported in the human infundibular nucleus in old men and postmenopausal women.^{114,115} The mean profile area of infundibular neurons from old men and postmenopausal women was significantly larger than that of young subjects (Figure 9). The density of hypertrophied neurons was also increased. Furthermore, the hypertrophied neurons contained increased amounts of neurokinin B, substance P,

and estrogen receptor mRNA, which indicated an increased neuronal activity as well.^{116,117} LHRH neurons are also found in this nucleus, but the hypertrophied neurons themselves do not contain this peptide. The neurokinin B-containing neurons are probably involved in the regulation of the HPG-axis and have been related to menopausal flushes.¹¹⁴ The sensitivity of LH to LHRH did not decrease in elderly males.¹¹⁸⁻¹²⁰ On the other hand, a decreased sensitivity to sex steroid feedback was found in elderly (80 years) postmenopausal women as compared to younger postmenopausal women (55 years).¹²¹ No data on changes in the exact number of LHRH expressing neurons in relation to aging in the human hypothalamus are available at present.

B. Growth Hormone-Releasing Hormone (GHRH) and Somatostatin

In the rat the majority of GHRH-immunoreactive neurons are located in the arcuate nucleus.^{122,123} Together with the inhibiting somatostatin neurons that are situated in the periventricular nucleus,¹²⁴ they regulate the secretion of growth hormone (GH), which is not only necessary for normal infant and childhood growth but also for the regulation of normal body composition and metabolism in adulthood.¹²⁵ A current hypothesis states that a defective GH secretion may be one of the pacemakers of aging.^{126,127} In addition, Morimoto¹²⁸ showed that the intensity of GHRH immunoreactivity in the median eminence of 20-month-old rats is markedly reduced in comparison with that of young rats. Interestingly, however, the number of immunoreactive neurons in the GHRH and somatostatin-containing neuronal perikarya in the hypothalamus in old colchicine-treated rats did not differ.¹²⁸ These observations indicate that in old animals GHRH and somatostatin-containing neurons remain present and have the capacity to synthesize the respective peptides. However, either the production, transport rate, or release of GHRH should be changed in aging. Indeed, De Gennaro Colonna et al.¹²⁹ reported a reduction in GHRH peptide and mRNA levels in the hypothalamus of (20-month) old male Sprague-Dawley rats. Both decreased somatostatin gene expression^{130,131} and increased somatostatin secretion have been reported in the rat.¹³² Endocrine studies indicate the presence of changes in GH regulation in human aging. In elderly people the response of GH to GHRH was shown to be significantly reduced.^{133,134} It has even been reported that GH responses to GHRH decline after 30 to 40 years in men and after menopause in women.¹³⁵⁻¹³⁷ Furthermore, the feedback effects of circulating GH on GHRH that are present in adult rats were not detectable in aged rats.¹²⁹ Although it has been suggested that a decreased GHRH or increased somatostatinergic activity are the main events underlying the age-related

decline of GH secretion,¹³⁸ no direct evidence is available about the changes of GHRH or somatostatin neurons in the human hypothalamus with aging.

VI. LATERAL TUBERAL NUCLEUS

The lateral tuberal nucleus (nucleus tuberalis lateralis, NTL) can only be recognized in man and higher primates. The only neuropeptide found so far that is characteristic for NTL neurons is somatostatin.^{9,124} In addition, some galanin and LHRH immunoreactive fibers have been found in the NTL.^{25,107} A dense innervation of prosomatostatin fibers, most probably derived from the NTL interneurons, were recently found in the NTL.¹²⁴ Receptors for CRH, somatostatin, muscarinic cholinergic receptors, benzodiazepin receptors, and *N*-methyl-D-aspartate (NMDA) receptors have been localized in the NTL.¹³⁹ In adulthood the NTL contains about 60,000 neurons. It has been shown that the number of NTL neurons may gradually decline with age. Although no significant correlation between NTL cell number and age was found, this may, however, be due to the small sample, especially in the oldest age group.¹³⁹ No clear changes in the somatostatin immunoreactive neurons were observed in human NTL with aging.¹²⁴

VII. CONCLUSION

The main conclusion of the present review is that the aging process causes differential changes in the hypothalamus. Aging may start around middle-age in some peptidergic systems (e.g., the decreased number of VIP neurons in middle-aged males and the sharp decline of SDN neurons after 50 years). On the other hand, many more systems remain perfectly intact and are even activated (e.g., the unchanged number of VIP neurons in the female SCN and the increased number of CRH and AVP neurons of the PVN in the course of aging). The latter pattern argues against the idea that normal aging leads to a general decline in cellular functions, i.e., by "wear and tear".

Activation of some groups of nerve cells within the physiological range seems to lead to maintenance of neurons during aging. This "use it or lose it" hypothesis might explain why certain neurons degenerate in aging while others do not, and why recovery of various neuronal systems during aging can be obtained by restoration of the missing stimulus.⁸⁸ The finding that some peptidergic neurons are activated during aging may have important functional consequences, e.g., the involvement of activated CRH neurons in the development of depression. In addition, the

presence of activated peptidergic neurons may have consequences for the treatment of elderly people with such peptides. When neuropeptides are given to elderly people, it should be taken into account that the release of many of them is already significantly increased.

Treatment of old and demented people with AVP and analogues was based upon the presumption that AVP levels in the brain were decreased,⁶⁵ whereas it was shown later that the endogenous activity of AVP cells in the HNS was increased in old people. It can be concluded that the multitude of changes in the various hypothalamic nuclei may be the basis for many functional changes in aging, i.e., both endocrine and central alterations. We suspect, however, that only a small proportion of such changes has, at present, been revealed.

ACKNOWLEDGMENTS

Brain material was obtained from the Netherlands Brain Bank, Amsterdam (coordinator Dr. R. Ravid). The authors are indebted to NUFFIC for financial support to Jiang-Ning Zhou, to Drs. M.A. Hofman, P. Lucassen, E. Van Someren, and A. Salehi for their excellent remarks, to Ms. W.T.P. Verweij for her secretarial help and for helping to revise the English, and to Mr. G. Van der Meulen for his photographic work.

REFERENCES

1. Brown-Séquard, C. R., Des effets produits chez l'homme par des injections souscutanées d'un liquide retiré des testicules de cobaye et de chien, *C.R. Soc. Biol.*, 41, 415, 1889.
2. Beach, F. A., Historical origins of modern research on hormones and behavior, *Horm. Behav.*, 15, 325, 1981.
3. Dilman, V. M., Age-associated elevation of hypothalamic threshold to feedback control, and its role in development, ageing and disease, *Lancet*, i, 1211, 1971.
4. Frolkis, V. V., Bezrukov, V. V., Duplenko, Y. K., and Genis, E. D., The hypothalamus in aging, *Exp. Gerontol.*, 7, 169, 1972.
5. Groen, J. J., General physiology of aging, *Geriatrics*, 14, 318, 1959.
6. Guillemin, R., Sakiz, E., and Ward, D. N., Further purification of TSH releasing from sheep hypothalamic tissues, with observations on the amino acid composition, *Proc. Soc. Exp. Biol. Med.*, 118, 1132, 1965.
7. Schally, A., Bowers, C., Redding, T., and Barrett, J., Isolation of thyrotropin releasing factor (TRF) from porcine hypothalamus, *Biochem. Biophys. Res. Commun.*, 25, 165, 1966.
8. Burgus, R. and Guillemin, R., Hypothalamic releasing factors, *Ann. Rev. Biochem.*, 39, 499, 1970.

9. Swaab, D. F., Hofman, M. A., Lucassen, P. J., Purba, J. S., Raadsheer, F. C., and Van de Nes, J. A. P., Functional neuroanatomy and neuropathology of the human hypothalamus, *Anat. Embryol. (Berl.)*, 187, 317, 1993.
10. Buijs, R. M., Chemical transmission in the brain. The role of amines, amino acid and peptides, in *Progress in Brain Research*, Buijs, R. M., Pévet, P. and Swaab, D. F., Eds., Elsevier, Amsterdam, 1983, 167.
11. Hökfelt, T., Johansson, O., Holets, V., Meister, T., and Melander, T., Distribution of neuropeptides with special reference to their coexistence with classical neurotransmitter, in *Psychopharmacology: The Third Generations of Progress*, Meltzer, H. Y., Ed., Raven Press, New York, 1987, 401.
12. Meites, J., Importance of the endocrine system in aging processes, *Adv. Biochem. Psychopharmacol.*, 43, 283, 1987.
13. Meites, J., Aging: hypothalamic catecholamines, neuroendocrine immune interactions and dietary restriction, *Proc. Soc. Exp. Biol. Med.*, 195, 304, 1990.
14. Horniekiewicz, O., Neurotransmitter changes in human brain during aging, in *Modification of Cell to Cell Signals During Normal and Pathological Aging*, Govani, S. and Battaini, F., Eds., Springer-Verlag, Berlin, 1987, 169.
15. Landfield, P., Baskin, R., and Pitler, T., Brain aging correlates: retardation by hormonal-pharmacological treatments, *Science*, 214, 581, 1981.
16. Sapolsky, R., Krey, L., and McEwen, B., Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging, *J. Neurosci.*, 5, 1222, 1985.
17. Bodansky, M. and Engel, S. L., Oxytocin and the life-span of male rats, *Nature*, 210, 751, 1966.
18. Fliers, E. and Swaab, D. F., Neuropeptide changes in aging and Alzheimer's disease, *Prog. Brain Res.*, 70, 141, 1986.
19. Rusak, B. and Zucker, I., Neural regulation of circadian rhythms, *Physiol. Rev.*, 59, 449, 1979.
20. Stopa, E. G., King, J. C., Lydic, R., and Schoene, W. C., Human brain contains vasopressin and vasoactive intestinal polypeptide neuronal subpopulations in the suprachiasmatic region, *Brain Res.*, 297, 159, 1984.
21. Swaab, D. F., Fliers, E., and Partiman, T. S., The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia, *Brain Res.*, 342, 37, 1985.
22. Mai, J. K., Kedziora, O., Teckhaus, L., and Sofroniew, M. V., Evidence for subdivisions in the human suprachiasmatic nucleus, *J. Comp. Neurol.*, 305, 508, 1991.
23. Moore, R. Y., The organization of the human circadian rhythm, the human hypothalamus in health and disease, in *Progress in Brain Research*, Vol. 93, Swaab, D. F., Hofman, M. A., Mirmiran, M., Ravid, R., and Van Leeuwen, F. W., Eds., Elsevier, Amsterdam, 1992, 101.
24. Albers, E., Liou, S., Stopa, E., and Zoeller, T., Interaction of colocalized neuropeptides: functional significance in the circadian timing system, *J. Neurosci.*, 11, 846, 1991.
25. Gai, W. P., Geffen, L. B., and Blessing, W. W., Galanin immunoreactive neurons in the human hypothalamus: colocalization with vasopressin-containing neurons, *J. Comp. Neurol.*, 298, 265, 1990.
26. Okamura, H., Murakami, S., Uda, K., Sugano, T., Takahashi, Y., Yanaihara, C., Yanahara, N., and Ibata, Y., Coexistence of vasoactive intestinal peptide (VIP), peptide histidine isoleucine amide (PHI), and gastrin releasing peptide (GRP)-like immunoreactivity in neurons of the rat suprachiasmatic nucleus, *Biomed. Res.*, 7, 295, 1986.

27. Francois-Bellan, A. M., Machidian, P., Dusticier, G., Tonon, M. C., Vaudry, H., and Bosler, O., GABA neurons in the rat suprachiasmatic nucleus: involvement in chemospecific circuitry and evidence for GAD-peptide colocalization, *J. Neurocytol.*, 19, 939, 1990.
28. Hofman, M. A. and Swaab, D. F., Seasonal changes in the suprachiasmatic nucleus of man, *Neurosci. Lett.*, 139, 257, 1992.
29. Hofman, M. A., Purba, J. S., and Swaab, D. F., Annual variations in the vasopressin neuron population of the human suprachiasmatic nucleus, *Neuroscience*, 53, 1103, 1993.
30. Hofman, M. A. and Swaab, D. F., Alterations in circadian rhythmicity of the vasopressin-producing neurons of the human suprachiasmatic nucleus (SCN) with aging, *Brain Res.*, 657, 281, 1994.
31. Van Gool, W. A. and Mirmiran, M., Aging and circadian rhythms, aging of the brain and Alzheimer's disease, *Progress in Brain Research*, Vol 70, Swaab, D. F., Fliers, E., Mirmiran, M., Van Gool, W. A., and Van Haaren, F., Eds., Elsevier, Amsterdam, 1986, 255.
32. Van Someren, E. J. W., Mirmiran, M., and Swaab, D. F., Non-pharmacological treatment of sleep and wake disturbances in aging and Alzheimer's disease: chronobiological perspectives, *Behavioral Brain Res.*, 57, 235, 1993.
33. Witting, W., Kwa, I. H., Eikelenboom, P., Mirmiran, M., and Swaab, D. F., Alterations in the circadian rest-activity rhythm in aging and Alzheimer's disease, *Biol. Psychiat.*, 27, 563, 1990.
34. Swaab, D. F., Zhou, J. N., Ehlhart, T., and Hofman, M. A., Development of vasoactive intestinal polypeptide neurons in the human suprachiasmatic nucleus in relation to birth and sex, *Dev. Brain Res.*, 79, 249, 1994b.
35. Zhou, J. N., Hofman, M. A., and Swaab, D. F., Vasoactive intestinal polypeptide neurons in the human suprachiasmatic nucleus in relation to sex, age and Alzheimer's disease, *Neurobiol. Aging*, (in press, 1995).
36. Davis, F. C., Darrow, J. M., and Menaker, M., Sex difference in the circadian control of hamster wheel-running activity, *Am. J. Physiol.*, 244, R93, 1983.
37. Wever, R. A., Characteristics of circadian rhythms in human function, *J. Neural. Transm.*, Suppl. 21, 323, 1986.
38. Van Der Beek, E. M., Wiegant, V. M., Van Der Donk, H. A., Van Den Hurk, R., and Buijs, R. M., Lesions of the suprachiasmatic nucleus indicate the presence of a direct vasoactive intestinal polypeptide-containing projection to gonadotrophin-releasing hormone neurons in the female rat, *J. Neuroendocrinol.*, 5, 137, 1993.
39. Swaab, D. F. and Hofman, M. A., An enlarged suprachiasmatic nucleus in homosexual men, *Brain Res.* 537, 141, 1990.
40. Swaab, D. F., Gooren, L. J. G., and Hofman, M. A., The human hypothalamus in relation to gender and sexual orientation, the human hypothalamus in health and disease, *Progress in Brain Research*, Vol. 93, Swaab, D. F., Hofman, M. A., Mirmiran, M., Ravid, R., and Van Leeuwen, F.W., Eds., Elsevier, Amsterdam, 1992, 205.
41. Hofman, M. A. and Swaab, D. F., Diurnal and seasonal rhythms of neuronal activity in the suprachiasmatic nucleus of humans, *J. Biol. Rhythms*, 8, 283, 1993a.
42. Rolandi, E., Franceshini, R., Cataldi, A., and Barreca, T., Twenty-four-hour secretory pattern of vasoactive intestinal polypeptide in the elderly, *Gerontology*, 36, 356, 1990.
43. Cugini, P., Lucia, P., Di Palma, L., Re, M., Leone, G., Battisti, P., Canova, R., Gasbarrone, L., and Cianetti, A., Vasoactive intestinal peptide fluctuates in human blood with a circadian rhythm, *Regul. Pept.*, 34, 141, 1991.

44. Prinz, P. N., Christie, C., Smallwood, R., Vitaliano, P., Bokan, J., Vitiello, M., and Martin, D., Circadian temperature variation in healthy aged and in Alzheimer's disease, *J. Gerontol.*, 39, 30, 1984.
45. Touitou, Y., Fèver, M., Lagoguey, M., Carayon, A., Bogdan, A., Reinberg, A., Beck, H., Cesselin, F., and Touitou, C., Age- and mental health-related circadian rhythms of plasma levels of melatonin, prolactin, luteinizing hormone and follicle-stimulating hormone in man, *J. Endocrinol.*, 91, 467, 1981.
46. Touitou, Y., Sulon, J., Bogdan, A., Touitou, C., Reinberg, A., Beck, H., Sodoyez, J., Demey-Ponsart, E., and Van Cauwenberghe, H., Adrenal circadian system in young and elderly human subjects: a comparative study, *J. Endocrinol.*, 93, 201, 1982.
47. Sherman, B., Wysham, C., and Pfohl, B., Age-related changes in the circadian rhythm of plasma cortisol in man, *J. Clin. Endocrinol. Metab.*, 61, 439, 1985.
48. Witting, W., Mirmiran, M., Bos, N. P. A., and Swaab, D. F., Effect of light intensity on diurnal sleep-wake distribution in young and old rats, *Brain Res. Bull.*, 30, 157, 1993.
49. Reuss, S. and Burger, K., Substance P-like immunoreactivity in the hypothalamic suprachiasmatic nucleus of *Phodopus sungorus* relation to daytime, photoperiod, sex and age, *Brain Res.*, 638, 189, 1994.
50. Braak, H. and Braak, E., The hypothalamus of the human adult: chiasmatic region, *Anat. Embryol.*, 176, 315, 1991.
51. Allen, L., Hines, M., Shryne, J., and Gorski, R., Two sexually dimorphic cell groups in the human brain, *J. Neurosci.*, 9, 497, 1989.
52. Swaab, D. F. and Hofman, M. A., Sexual differentiation of the human hypothalamus ontogeny of the sexually dimorphic nucleus of the preoptic area, *Dev. Brain Res.*, 44, 314, 1988.
53. Swaab, D. F. and Fliers, E., A sexually dimorphic nucleus in the human brain, *Science*, 228, 1112, 1985.
54. Hofman, M. A. and Swaab, D. F., The sexually dimorphic nucleus of the preoptic area in the human brain: a comparative morphometric study, *J. Anat.*, 164, 55, 1989.
55. Vermeulen, A., Androgens and male senescence, in *Testosterone: Action, Deficiency, Substitution*, Nieschlag, E. and Behre, H. M., Eds., Springer-Verlag, New York, 1990, 629.
56. Fliers, E., Noppen, N. W. A. M., Wiersinga, W. M., Visser, T., and Swaab, D. F., Distribution of thyrotropin-releasing hormone (TRH)-containing cells and fibers in the human hypothalamus, 350, 311, 1994.
57. Simerly, R. B., Gorski, R. A., and Swanson, L. W., Neurotransmitter specificity of cells and fibers in the medial preoptic nucleus: an immunohistochemical study in the rat, *J. Comp. Neurol.*, 246, 343, 1986.
58. Handler, J. S. and Orlof, J., Antidiuretic hormone, *Ann. Rev. Physiol.*, 43, 611, 1981.
59. Cowley, A. W. and Barber, B. J., Vasopressin vascular and reflex effects — a theoretical analysis, the neurohypophysis: structure, function and control, *Progress in Brain Research*, Cross, B. A. and Leng, G., Eds., Elsevier, Amsterdam, 1983, 415.
60. Swaab, D. F. and Boer, K., Function of pituitary hormones in human parturition — a comparison with data in the rat, in *Human Parturition*, Keirse, M. J. N. C., Anderson, A. B. M., and Gravenhorst, B. J., Eds., Martinus Nijhoff, The Hague, 1979, 49.
61. Spencer, S., Saper, C. B., Joh, T., Reis, D. J., Goldstein, M., and Raese, J. D., Distribution of catecholamine-containing neurons in the normal human hypothalamus, *Brain Res.*, 328, 730, 1985.

62. Panayotacopoulou, M. and Swaab, D. F., Development of tyrosine hydroxylase-immunoreactive neurons in the human paraventricular and supraoptic nucleus, *Dev. Brain Res.*, 72, 145, 1993.
63. De Wied, D., Central actions of neurohypophysial hormones, the neurohypophysis: structure, function and control, in *Progress in Brain Research*, Cross, B. A. and Leng, G., Eds., Elsevier, Amsterdam, 1985, 155.
64. De Wied, D. and Van Ree, J. M., Neuropeptides: animal behaviour and human psychopathology, *Eur. Arch. Psychiatry Neurol. Sci.*, 238, 323, 1989.
65. Jolles, J., Neuropeptides and the treatment of cognitive deficits in aging and dementia, aging of the brain and Alzheimer's disease, in *Progress in Brain Research*, Swaab, D. F., Fliers, E., Mirmiran, M., Van Gool, W. A., and Van Haaren, F., Eds., Elsevier, Amsterdam, 1986, 429.
66. Carter, L. S., Oxytocin and sexual behavior, *Neurosci. Biobehav. Rev.*, 16, 131, 1992.
67. Insel, T. R., Oxytocin — a neuropeptide for affiliation: evidence from behavioral, receptor autoradiographic, and comparative studies, *Psychoneuroendocrinology*, 17, 3, 1992.
68. Murphy, M. R., Seckl, J. R., Burton, S., Checkley, S. A., and Lightman, S. L., Changes in oxytocin and vasopressin secretion during sexual activity in men, *J. Clin. Endocrinol. Metab.*, 65, 738, 1987.
69. Goudsmit, E., Neijmeijer-Leloux, A., and Swaab, D. F., The human hypothalamo-neurohypophyseal system in relation to development, aging and Alzheimer's disease, *Prog. Brain Res.*, 93, 237, 1992.
70. Fliers, E., Swaab, D. F., Pool, C. W., and Verwer, R. W., The vasopressin and oxytocin neurons in the human supraoptic and paraventricular nucleus: changes with aging and in senile dementia, *Brain Res.*, 342, 45, 1985.
71. Vogels, O. J., Broere, C. A., and Nieuwenhuys, R., Neuronal hypertrophy in the human supraoptic and paraventricular nucleus in aging and Alzheimer's disease, *Neurosci. Lett.*, 109, 62, 1990.
72. Hoogendijk, J. E., Fliers, E., Swaab, D. F., and Verwer, R. W., Activation of vasopressin neurons in the human supraoptic and paraventricular nucleus in senescence and senile dementia, *J. Neurol. Sci.*, 69, 291, 1985.
73. Hofman, M. A., Fliers, E., Goudsmit, E., and Swaab, D. F., Morphometric analysis of the suprachiasmatic and paraventricular nuclei in the human brain: sex differences and age-dependent changes, *J. Anat.*, 160, 55, 1988.
74. Hofman, M. A., Goudsmit, E., Purba, J. S., and Swaab, D. F., Morphometric analysis of the supraoptic nucleus in the human brain, *J. Anat.*, 172, 259, 1990.
75. Goudsmit, E., Hofman, M. A., Fliers, E., and Swaab, D. F., The supraoptic and paraventricular nuclei of the human hypothalamus in relation to sex, age and Alzheimer's disease, *Neurobiol. Aging*, 11, 529, 1990.
76. Van der Woude, P. F., Goudsmit, E., Wierda, M., Purba, J. S., Hofman, M. A., Bogte, H., and Swaab, D. F., No vasopressin cell loss in the human hypothalamus in aging and Alzheimer's disease, *Neurobiol. Aging*, (in press, 1995).
77. Lucassen, P. J., Ravid, R., Gonatas, N. K., and Swaab, D. F., Activation of the human supraoptic and paraventricular nucleus neurons with aging and in Alzheimer's as judged from increasing size of Golgi apparatus, *Brain Res.*, 632, 105, 1993.
78. Phillips, P. A., Johnston, C. I., and Gray, L., Disturbed fluid and electrolyte homeostasis following dehydration in elderly people, *Age & Ageing*, 22, S26, 1993.
79. Kirkland, J., Lye, M., Goddard, C., Vargas, E., and Davies, I., Plasma arginine vasopressin in dehydrated elderly patients, *Clin. Endocrinol.*, 20, 451, 1984.
80. Helderman, J. H., Vestal, R. E., Rowe, J. W., Tobin, J. D., Andres, R., and Robertson, G. L., The response of arginine vasopressin to intravenous ethanol and hypertonic saline in man, the impact of aging, *J. Gerontol.*, 33, 39, 1978.

81. Rowe, J. W., Minaker, K. L., Sparrow, D., and Robertson, G. L., Age-related failure of volume-pressure-mediated vasopressin release, *J. Clin. Endocrinol. Metab.*, 54, 661, 1982.
82. Crawford, G. A., Johnson, A. G., Gyory, A. Z., and Kelly, D., Change in arginine vasopressin concentrations with age [letter], *Clin. Chem.*, 39, 2023, 1993.
83. Faull, C. M., Holmes, C., and Baylis, P. H., Water balance in elderly people: is there a deficiency of vasopressin?, *Age & Ageing*, 22, 114, 1993.
84. Burszty, M., Bresnahan, M., Gavras, I., and Gavras, H., Effect of aging on vasopressin, catecholamines, and alpha 2-adrenergic receptors, *J. Am. Geriatr. Soc.*, 38, 628, 1990.
85. Ravid, R., Fliers, E., Swaab, D.F., and Zurcher, C., Changes in vasopressin and testosterone in the senescent Brown-Norway (BN/BiRij) rat, *Gerontology*, 33, 87, 1987.
86. Herzberg, N.H., Goudsmit, E., Kruisbrink, J., and Boer, G. J., Testosterone treatment restores reduced vasopressin binding sites in the kidney of the aging rat, *J. Endocrinol.*, 123, 59, 1989.
87. Wierda, M., Goudsmit, E., Van der Woude, P. F., Purba, J. S., Hofman, M. A., Bogte, H., and Swaab, D. F., Oxytocin cell number in the human paraventricular nucleus remains constant with aging and in Alzheimer's disease, *Neurobiol. Aging*, 12, 511, 1991.
88. Swaab, D.F., Brain aging and Alzheimer's disease, "wear and tear" versus "use it or lose it", *Neurobiol. Aging*, 12, 317, 1991.
89. Pelletier, G., Desy, L., Cote, J., and Vaudry, H., Immunocytochemical localization of corticotropin-releasing factor-like immunoreactivity in the human hypothalamus, *Neurosci. Lett.*, 41, 259, 1983.
90. Raadsheer, F. C., Sluiter, A. A., Ravid, R., Tilders, F. J., and Swaab, D. F., Localization of corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus of the human hypothalamus; age-dependent colocalization with vasopressin, *Brain Res.*, 615, 50, 1993.
91. Merchenthaler, I., Vigh, S., Petrusz, P., and Schally, A. V., Immunocytochemical localization of corticotropin-releasing factor (CRF) in the rat brain, *Am. J. Anat.*, 165, 385, 1982.
92. Swanson, L., Sawchenko, P. E., and Lind, R. W., Regulation of multiple peptides in CRF parvicellular neurosecretory neurons: implications for the stress response, *Prog. Brain Res.*, 68, 169, 1986.
93. Sawchenko, P. F. and Swanson, L. W., Organization of CRF immunoreactive cells and fibers in the rat brain; immunohistochemical studies, in *Corticotropin Releasing Factor: Basic and Clinical Studies of a Neuropeptide*, De Souza, E. B. and Nemeroff, C. B., Eds., CRC Press, Boca Raton, FL, 1990, 29.
94. Raadsheer, F. C., Oorschot, D. F., Verwer, R. W. H., Tilders, F. J. H., and Swaab, D. F., Age-related increase in the total number of corticotropin-releasing hormone neurons in the human paraventricular nucleus in controls and Alzheimer's disease: comparison of the disector with an unfolding method, *J. Comp. Neurol.*, 339, 447, 1994.
95. Swaab, D. F., Raadsheer, F. C., Endert, E., Hofman, M. A., Kamphorst, W., and Ravid, R., Increased cortisol levels in aging and Alzheimer's disease in postmortem cerebrospinal fluid, *J. Neuroendocrinol.*, 6, 681, 1994.
96. Dodt, C., Dittman, J., Hruby, J., Späth-Schwalbe, E., Born, J., Schüttler, R., and Fehm, H. L., Different regulation of adrenocorticotropin and cortisol secretion in young, mentally healthy elderly, and patients with senile dementia of Alzheimer's type, *J. Clin. Endocrinol. Metab.*, 72, 272, 1991.
97. Oxenkrug, G. F., Pomara, N., McIntyre, I. M., Brancinnier, R. J., Stanly, M., and Gershon, S., Aging and cortisol resistance to suppression by dexamethasone: a positive correlation, *Psychiatry Res.*, 10, 125, 1983.

98. Rosenbaum, A. H., Schatzberg, A. F., MacLaughlin, R. A., Snyder, K., Jiang, N. S., Ilstrup, D., Rothschild, A. J., and Kliman, B., The dexamethasone suppression test in normal control subjects: comparison of two assays and effect of age, *Am. J. Psychiatry*, 141, 1550, 1984.
99. Herman, J. P., Schafer, M. K. H., Young, E. A., Thompson, R., Douglass, J., Akil, H., and Watson, S. J., Evidence for hippocampal regulation of neuroendocrine neurons of the hypothalamo-pituitary-adrenocortical axis, *J. Neurosci.*, 9, 3072, 1989.
100. Blichert-Toft, M., Secretion of corticotrophin and somatotrophin by the senescent adenohypophysis in the man, *Acta Endocrinol.*, 78 (Suppl. 195), 15, 1975.
101. Holsboer, F., Psychiatric implications of altered limbic-hypothalamic-pituitary-adrenocortical activity, review, *Eur. Arch. Psychiatry Neurol. Sci.*, 238, 302, 1989.
102. Pekary, A. E., Carlson, H. E., Tadataka, Y., Sharp, B., Walfish, P. G., and Hershman, J. M., Thyrotropin-releasing hormone levels decrease in hypothalamus of aging rats. *Neurobiol. Aging*, 5, 221, 1984.
103. Pekary, A. E., Mirell, C. J., Turner, L. F., Walfish, P. G., and Hershman, J. M., Hypothalamic secretion of thyrotropin releasing hormone decline in aging rats, *J. Gerontol.*, 42, 447, 1987.
104. Spaulding, S. W., Age and the thyroid. *Endocrinol. Metab. Clin.*, 16, 1013, 1987.
105. Simpkins, J. W. and Millard, W. J., Influence of age on neurotransmitter function, *Endocrinol. Metab. Clin. North. Am.*, 16, 893, 1987.
106. Bouras, C., Magistretti, J., Morrison, H., and Constantinidis, J., An immunohistochemical study of pro-somatostatin-derived peptides in the human brain, *Neuroscience*, 22, 781, 1987.
107. Najimi, M., Chigr, F., Jordan, D., Leduque, P., Block, B., Tommasi, M., Rebaud, P., and Kopp, N., Anatomical distribution of LHRH-immunoreactive neurons in the human infant hypothalamus and extrahypothalamic regions, *Brain Res.*, 516, 280, 1990.
108. Mezey, E. and Kiss, J. Z., Vasoactive intestinal peptide-containing neurons in the paraventricular nucleus may participate in regulating prolactin secretion, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 245, 1985.
109. Anthony, E. L., King, J. C., and Stopa, E. G., Immunocytochemical localization of LHRH in the median eminence, infundibular stalk, and neurohypophysis. Evidence for multiple sites of releasing hormone secretion in human and other mammals, *Cell Tissue Res.*, 236, 5, 1984.
110. Barry, J., Characterization and topography of LHRH neurons in the human brain, *Neurosci. Lett.*, 3, 287, 1976.
111. Stopa, E. G., Koh, E. T., Svendsen, C. N., Rogers, W. T., Schwaber, J. S., and King, J. C., Computer-assisted mapping of immunoreactive mammalian gonadotropin-releasing hormone in adult human basal forebrain and amygdala, *Endocrinology*, 128, 3199, 1991.
112. Rance, N. E., Young, S., III, and McMullen, N., Topography of neurons expressing luteinizing hormone-releasing hormone gene transcripts in the human hypothalamus and basal forebrain, *J. Comp. Neurol.*, 339, 573, 1994.
113. Vallet, P. G., Charnay, Y., and Bouras, C., Distribution and colocalization of delta sleep-inducing peptide and luteinizing hormone-releasing hormone in the aged human brain: an immunohistochemical study, *J. Chem. Neuroanat.*, 3, 207, 1990.
114. Rance, N. E., Hormonal influence on morphology and neuropeptide gene expression in the infundibular nucleus of postmenopausal women, in *Progress in Brain Research*, Swaab, D. F., Hofman, M. A., Mirmiran, M., Ravid, R., and Van Leeuwen, F. W., Eds., Elsevier, Amsterdam, 1992.
115. Rance, N. E., Uswandi, S. V., and McMullen, N. T., Neuronal hypertrophy in the older men, *Neurobiol. Aging*, 14, 337, 1993.

116. Rance, N. E., McMullen, N. T., Smialek, J. E., Price, D. L., and Young, W. S., III, Postmenopausal hypertrophy of neurons expressing the estrogen receptor gene in the human hypothalamus, *J. Clin. Endocrinol. Metab.*, 71, 79, 1990.
117. Rance, N. E. and Young, W. S., III, Hypertrophy and increased gene expression of neurons containing neurokinin B and substance P messenger RNAs in the hypothalami of postmenopausal women, *Endocrinology*, 128, 2239, 1991.
118. Giusti, M., Marini, G., Traverso, L., Cavagnaro, P., Granziera, L., and Giordano, G., Effect of pulsatile luteinizing hormone-releasing hormone administration on pituitary-gonadal function in elderly man, *J. Endocrinol. Invest.*, 13, 127, 1990.
119. Kaufman, J. M., Deslypere, J. P., Giri, M., and Vermeulen, A., Neuroendocrine regulation of pulsatile luteinizing hormone secretion in elderly men, *J. Steroid Biochem. Mol. Biol.*, 37, 421, 1990.
120. Kaufman, J. M., Giri, M., Deslypere, J. M., Thomas, G., and Vermeulen, A., Influence of age on the responsiveness of the gonadotrophs to luteinizing hormone-releasing hormone in males, *J. Clin. Endocrinol. Metab.*, 72, 1255, 1991.
121. Rossmanith, W., Reichelt, C., and Scherbaum, W., Neuroendocrinology of aging in humans: attenuated sensitivity to sex steroid feedback in elderly postmenopausal women, *Neuroendocrinology*, 59, 355, 1994.
122. Bloch, B., Ling, N., Benoit, R., Wehernberg, W. B., and Guillemin, R., Specific depletion of immunoreactive growth hormone releasing factor by monosodium glutamate in rat median eminence, *Nature*, 307, 272, 1984.
123. Liposits, Z., Hrabovszky, E., and Paull, W. K., Catecholaminergic afferents to growth hormone-releasing hormone (GHRH)-synthesizing neurons of the arcuate nucleus in the rat, *Biomed. Res.*, 40 (Suppl. 3), 1950, 1989.
124. Van de Nes, J. A. P., Sluiter A.A., Pool, C. W., Kamphorst, W., Ravid, R., and Swaab, D. F., The monoclonal antibody Alz-50, which reveals cytoskeletal changes in Alzheimer's disease, cross-reacts with somatostatinergic neurons in the human hypothalamus of control subjects, *Brain Res.*, 655, 97, 1994.
125. Christiansen, J. S., Jorgensen, J. O., Pedersen, S.A., Moller, J., Jorgensen, J., and Kakkebaek, N. E., Effects of growth hormone on body composition in adults, *Horm. Res.*, 33 (Suppl. 4), 61, 1990.
126. Rudman, D., Kutner, M. H., Rogers, C. M., Lubin, M. F., Fleming, G. H., and Bain, R. P., Impaired growth hormone secretion in the adult population, *J. Clin. Invest.*, 67, 1361, 1981.
127. Rudman, D., Feller, A. G., Nagraj, H. S., Gergans, G. A., Lalitha, P. Y., Goldberg A. F., Schlenker, R. A., Cohn, L., Rudman, I., and Mattson, D. E., Effect of human growth hormone in men over 60 years old, *New Engl. J. Med.*, 323, 1, 1990.
128. Morimoto, N., Kawakami, F., Makino, S., Chihara, K., Hasegawa, M., and Ibata, Y., Age-related changes in growth hormone releasing factor and somatostatin in the rat hypothalamus, *Neuroendocrinology*, 47, 459, 1988.
129. De Gennaro Colonna, V., Fidone, F., Cocchi, D., and Müller, E. E., Feedback effects of growth hormone to growth hormone-releasing hormone and somatostatin are not evident in aged rats, *Neurobiol. Aging*, 14, 503, 1993.
130. Sonntag, W. E., Boyd, R. L., and Booze, R. M., Somatostatin gene expression in hypothalamus and cortex of aging male rats, *Neurobiol. Aging*, 11, 409, 1990.
131. Martinoli, M. C., Ouellet, J., Rhéaume, E., and Pelletier, G., Growth hormone and somatostatin gene expression as measured by quantitative in situ hybridization, *Neuroendocrinology*, 54, 607, 1991.
132. Ferrara, C., Ceresoli, G., Marcezzi, C., and Cocchi, D., Hypothalamic-pituitary somatostatinergic system in middle-aged female rats, *Hormone Metab. Res.*, 23, 243, 1991.

133. Coiro, V., Volpi, R., Cavazzini, U., Bertoni, P., Corradi, A., Bianconi, L., Davoli, C., Rossi, G., and Chiodera, P., Restoration of normal growth hormone responsiveness to GHRH in normal aged men by infusion of low amounts of theophylline [published erratum appears in *J. Gerontol.*, 47(2), M34, 1992], *J. Gerontol.*, 46, M155, 1991.
134. Coiro, V., Volpi, R., Bertoni, P., Finzi, G., Marcato, A., Caiazza, A., Colla, R., Giacalone, G., Rossi, G., and Chiodera, P., Effect of potentiation of cholinergic tone by pyridostigmine on the GH response to GHRH in elderly men, *Gerontology*, 38, 217, 1992.
135. Finkelstein, J. W., Rottwarg, H. P., Boyar, R. M., Kream, J., and Hellman, L. Age-related changes in 24-hour spontaneous secretion of growth hormone, *J. Clin. Endocrinol.*, 35, 665, 1972.
136. Carlson, H. E., Gillan, J. C., Gordon, P., and Snyder, F., Absence of sleep-related growth peaks in aged normal subjects and acromegaly, *J. Clin. Endocrinol.*, 34, 1102, 1972.
137. Iranmanesh, A., Lizarralde, G., and Veldhuis, J. D., Age and relative adiposity are specific negative determinants of the frequency and amplitude of growth hormone (GH) secretory bursts and the half-life of endogenous GH in healthy men, *J. Clin. Endocrinol. Metab.*, 73, 1081, 1991.
138. Müller, E. E., Cella, S. G., de Gennaro Colonna, V., Parenti, M., Cocchi, D., and Locatelli, V., Aspects of the neuroendocrine control of growth hormone secretion in aging mammals, *J. Reprod. Fertil. Suppl.*, 46, 99, 1993.
139. Kremer, H. P., The hypothalamic lateral tuberal nucleus: normal anatomy and changes in neurological diseases, *Prog. Brain Res.*, 93, 249, 1992.
140. Swaab, D. F., Roozendaal, B., Ravid, R., Velis, D. N., Gooren, L., and Williams, R. S., Suprachiasmatic nucleus in aging, Alzheimer's disease, transsexuality and Prader-Willi syndrome, in neuropeptides and brain function, *Progress in Brain Research*, Vol. 72, De Kloet, R. et al., Eds., Elsevier, Amsterdam, 1987, 301.