

RITA JÓZSA – BÉLA MESS

LOCALIZATION
AND ONTOGENY OF
PEPTIDERGIC NEURONS
IN BIRDS

COMPARATIVE STUDIES ON THE RELEASING AND
INHIBITING HORMONES IN BIRDS AND MAMMALS

AKADÉMIAI KIADÓ, BUDAPEST

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in Birds and Mammals

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TUDOMÁNYOS AKADEMIA
KÖNYVTÁRA

ISBN 963 05 6608 7

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Akadémiai Kiadó, H-1519 Budapest, P.O. Box 245

Typesetting by Fényszedő Központ Kft.

Printed in Hungary
by Akadémiai Kiadó és Nyomda Vállalat, Budapest

PREFACE

Our interest has been focussed on the topic of the localization of the releasing and inhibiting hormone-producing neuron system since the mid-60s. In collaboration with Dr. Martini's group in Milan, we have tried to map the intrahypothalamic localization of the thyrotropin-releasing hormone (TRH), the follicle stimulating hormone-releasing hormone (FSH-RH), the luteinizing hormone-releasing hormone (LHRH) and the corticotropin-releasing factor (CRF) in the rat brain (Mess et al. 1967). On the other hand, the ontogenetic differentiation of the hypophyseal-target organ system in chicken embryos has also been systematically investigated from the late 60s. The results of these investigations have been summarized in a short monograph (Mess and Straznicky 1970). All these studies, using different hormone bioassays, morphological techniques (autoradiography, electron microscopy) or physiological methods (thyroid iodide uptake and release) have not provided unequivocal data concerning the precise localization and specific identification of the different releasing and inhibiting hormone-containing neuronal elements.

The immunocytochemical techniques, established by Dr. Sétáló in our laboratory in 1973, have opened up new vistas also in our studies. A research group in our laboratory succeeded in mapping LHRH- and CRF-immunopositive neuron systems and pathways in mammals (Flerkó et al. 1980, Merchenthaler et al. 1984, Vigh et al. 1984). Later, we described the localization of TRH-immunopositive cells and pathways in the rat brain (Merchenthaler et al. 1988).

On the basis of our previous studies and in order to present a new methodological approach, it seemed worthwhile investigating the localization of the different releasing and inhibiting hormone-producing neuron systems in the avian brain. This approach has provided the possibility to conduct direct comparative studies between birds and mammals within the same laboratory.

Immunocytochemical techniques have also been used in our laboratory for developmental endocrinological studies of the releasing hormone systems in mammals (Horváth et al. 1982). Therefore, we did not want to omit the possibil-

ity of making a direct comparison between birds and mammals also from the developmental endocrinological aspect.

The present book comprises the main results of over 10 years of our research work devoted to avian endocrinology. We wish to emphasize again that the main goal of these investigations was to examine the different peptidergic systems from a comparative endocrinological point of view. We are convinced that comparative data may help in a better understanding of several neuroendocrine mechanisms.

Since this book is entirely based on immunocytochemical studies, this work has been scheduled to approach the form of a histological atlas. Therefore, about 50% of the total volume is devoted to microphotographs and schematic drawings representing recapitulations of some important relationships. When appropriate, we tried to summarize some comparative data also in a few tables. Therefore, the text was subordinated in some respect to the Figures and Tables. References were also restricted, especially in the case of the mammalian data, to some representative reports.

The authors wish to express their deep appreciation to the Biological Section of the Hungarian Academy of Sciences for the financial support of their experimental work. They are also deeply indebted to the Hungarian National Scientific Research Foundation (OTKA) for sponsoring their investigations. They acknowledge the Hungarian Academy of Sciences for providing the opportunity to publish this short monograph.

This work could not have been completed without the kind cooperation of nearly all members of our Department. We are grateful to Professor B. Flerkó (previous head of the Department of Anatomy, University Medical School, Pécs) for his continued interest in our work. Special thanks are due to Dr. G. Sétáló (present head of our department) for introducing immunocytochemical methods in our department and to his research group (Drs S. Vigh, I. Merchenthaler and J. Horváth) for placing their data, obtained in mammals, at our disposal for comparison.

We wish to express our sincere gratitude to the following scientists and institutions for supplying us with antisera or antigens to generate a specific antiserum: to the late Dr. L. Kisfaludy (Gedeon Richter Pharmaceutical Works, Kőbánya, Budapest) for synthesizing a special TRH-acid for preparing TRH-antigen; to Dr. V. Csernus (our department) for generating TRH-antiserum; to Dr. S. Vigh for generating CRF-antiserum in the Department of Medicine, Tulane University, USA; to Drs G. Sétáló and J. Horváth for LHRH (our department); to T. Görcs (Neurobiology-Research Group of the Hungarian Academy of Sciences, 1st Department of Anatomy, Semmelweis University Medical School, Budapest), P. Petrusz (Department of Cell Biology and Anatomy, University of North Carolina, Chapel Hill, USA) for preparing SRIF-antiserum; and to Dr. C.G. Scanes (Department of Animal Sciences, Rutgers

University, New Brunswick, USA) for donation of antisera against avian pituitary tropic hormones.

We are further indebted to Professor A. Oksche and Dr. H. W. Korf (Department of Anatomy, Justus Liebig University of Giessen, Germany) for their valuable scientific and financial aid. Studies on the localization of TRH were performed in their department.

The valuable technical assistance of the following members of our Department is sincerely acknowledged: Mrs D. Ömböli-Pintér for her skillful histotechnical assistance; Ms M. Soltész for preparing the photographs; Ms M. Szelier for designing the schemes and drawings, and Mrs Bágyi for typing the manuscript. Last, but not least, it is the pleasure of the authors to acknowledge Dr. Alan Corbin's (Wyeth Ayerst-Research, Princeton, New Jersey, USA) kind linguistic supervision of their manuscript.

The Authors

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LIST OF ABBREVIATIONS*

Rat

AAA	anterior amygdaloid area
A5	numbered catecholaminergic cell groups
ABL	basolateral amygdaloid nucleus
ACE	central amygdaloid nucleus
ACO	anterior cortical nucleus
AME	medial amygdaloid nucleus
AON	anterior olfactory nucleus
ARC	arcuate nucleus
BNST	bed nucleus of the stria terminalis
CA	anterior commissure
CC	corpus callosum
DMN	dorsomedial nucleus
XI	nucleus nervi accessorii
GR	nucleus gracilis
HI	hippocampus
IC	inferior colliculus
IP	interpenduncular nucleus
LC	locus coeruleus
LRN	lateral reticular nucleus
LV	lateral ventricle
MM	medial mammillary nucleus
MPOA	medial preoptic area
NRGC	nucleus reticularis gigantocellularis
NRPC	nucleus reticularis parvocellularis

NVM	medial vestibular nucleus
OB	olfactory bulb
PAG	periaqueductal gray
PB	parabrachial nucleus
PIR	piriform cortex
PVN	paraventricular nucleus
RF	reticular formation
RM	raphe magnus
RtTG	reticulotegmental nucleus of the pons
SNC	substantia nigra
SOL	solitary tract
SON	supraoptic nucleus
X	nucleus nervi vagi
TH	thalamus
TO	optic tract
Tu	olfactory tubercle
III	nucleus nervi oculomotorii

Chicken

Ac	nucleus accumbens
AHR	area hypothalami posterioris
AM	nucleus anterior medialis hypothalami
BO	olfactory bulb
CA	anterior commissure
Cb	cerebellum
CO	optic chiasm

* *Note:* This list (being valid for both rat and chicken) gives the abbreviations of Figs 14-17 and 28-29.

CP	posterior commissure	OVL	organum vasculosum laminae terminalis
CT	tectal commissure	PA	palaeostriatum augmentatum
DLAm	nucleus dorsolateralis anterior thalami, pars medialis	PHN	nucleus periventricularis hypothalami
DMA	nucleus dorsomedialis anterior thalami	POA	nucleus preopticus anterior
DMP	nucleus dorsomedialis posterior thalami	POM	nucleus preopticus medialis
FDB	diagonal fascicle of Broca	POP	nucleus preopticus periventricularis
FLM	fasciculus longitudinalis medialis	PVN	paraventricular nucleus
GCT	central gray of the mesencephalon	PVO	periventricular organ
HA	hyperstriatum accessorium	R	raphe nucleus
Hb	habenular nucleus	Rpc	nucleus reticularis parvocellularis
Hp	hippocampus	RPgc	nucleus reticularis pontis caudalis
ICo	intercollicular nucleus	S	nucleus tractus solitarii
IH	nucleus inferioris hypothalami	SCE	stratum cellulare externum
IN	nucleus infundibuli hypothalami	SCI	stratum cellulare internum
IP	interpenduncular nucleus	SCN	suprachiasmatic nucleus
LHy	regio lateralis hypothalami	SL	nucleus septalis lateralis
LoC	locus coeruleus	SM	nucleus septalis medialis
LPO	parolfactory lobe	SOv	nucleus supraopticus, pars ventralis
MM	mammillary nucleus	X	nucleus nervi vagi
MN V	nucleus mesencephalicus nervi trigemini	III	nucleus nervi oculomotorii
MPO	nucleus magnocellularis preopticus	Tn	temporal nucleus
(d)	pars dorsalis	TO	nucleus taeniae
(m)	pars medialis	TSM	septomesencephalic tract
NIII	oculomotor nerve	Tu	tuberal nucleus
OM	nucleus nervi oculomotorii	V	third ventricle
(d)	pars dorsomedialis	VL	lateral ventricle
(v)	pars ventralis	VMN	nucleus ventromedialis hypothalami

Chapter 1. SURVEY OF THE LITERATURE

Localization of the Releasing and Inhibiting Hormone-producing Neurons in Mammals and Birds

Mammals

Before providing a brief review of the literature dealing with the site of production of the different releasing hormones (RHs) and inhibiting hormones (IHs), a general commentary would be appropriate. At present, there is a considerable confusion regarding the nomenclature of these neurosecretory materials. Some authors prefer the terminology "releasing or inhibiting hormones" indicating that these are real hormones produced by neurons of the central nervous system. Others use the term "releasing and inhibiting factors" arguing that these substances do not fulfil all the theoretical requirements (including structural identification) of a hormone. Besides this general discrepancy, the nomenclature is further confused by using different names for the same substance. Luteinizing hormone-releasing hormone (LHRH) is often called luteinizing hormone-releasing hormone (LHRH) or gonadotropin-releasing hormone (GnRH). Since there is no convincing evidence in the literature indicating the existence of FSH-RH and LHRH separately in birds, we used the term "LHRH" as a representative of GnRH. Similarly, as in case of the somatotropin release-inhibiting factor (SRIF), the term "somatostatin" is often used. It is not our duty to take a stand in this theoretical debate. For practical reasons we adopted this illogical custom that is widely accepted in the literature. Thyrotropin-releasing hormone (TRH) and LHRH are termed "hormones", while corticotropin-releasing factor (CRF) and SRIF are termed "factors".

After the formulation of the fundamental neuroendocrine concept of Harris (Harris 1955) suggesting the existence of neurohormones synthesized by hypothalamic neurons playing a key role in the neuroendocrine regulation exerted by the hypothalamo-hypophyseal axis, the existence of several releasing hormones were proposed within a relatively short time. The sequence of the description of the different releasing and inhibiting hormones is indicated in *Table I* (Mess et al. 1970). The majority of these neuropeptides had still not been isolated and chemically identified, when many laboratories, including ours, made attempts to find the localization of neurons producing these peptides. More or less indirect methods were applied to determine the site of production

Table I
Hypothalamic releasing and inhibiting factors

Hypothalamic factors	Abbreviations	References
Corticotropin-Releasing Factor	CRF	Saffran et al. (1955)
Fractions of CRF	α_1 -CRF	Guillemin et al. (1960)
	α_2 -CRF	Schally et al. (1962)
	β -CRF	Schally et al. (1962)
Thyrotropin-Releasing Factor	TSHRF	Shibusawa et al. (1959)
Luteinizing Hormone-Releasing Factor	LHRF	McCann et al. (1960)
Growth Hormone-Releasing Factor	GHRF	Franz et al. (1962)
Follicle Stimulating Hormone-Releasing Factor	FSHRF	Igarashi and McCann (1964)
Melanocyte Stimulating Hormone-Releasing Factor	MSHRF	Taleisnik and Orias (1965)
Prolactin-Releasing Factor (pigeon)	PRF	Kragt and Meites (1965)
Prolactin-Inhibiting Factor (mammals)	PIF	Pasteels (1962)
Melanocyte Stimulating Hormone-Inhibiting Factor	MSHIF	Kastin (1965)
Growth Hormone-Inhibiting Factor	GHIF	Krulich et al. (1967)

of these supposed neurohormones. Watanabe and McCann (1968) used serial frozen sections of the hypothalamus, the releasing hormones were extracted from these slides and were tested for releasing hormone activity. Palkovits and co-workers (1974) applied punching techniques for the same purpose. We attempted to obtain more substantial information in this field by placing well-defined lesions into different areas of the hypothalamus. The loss of the examined releasing hormone activity in the extract of the lesioned hypothalamus served for mapping the site of production of the given peptide (Mess et al. 1967, 1970). Data obtained in different laboratories using divergent techniques as mentioned above, were fairly contradictory in details. However, it was generally accepted that TRH neurons (Guillemin et al. 1965, Averill and Kennedy 1967) and LHRH neurons (McCann 1962, Endr czi and Hilliard 1965, Barry et al. 1973) appear to have the widest distribution in the hypothalamus. These data were also supported by our findings (Mess et al. 1967). The majority of the authors (Brizze and Eik-Nes 1961, Porter 1968) agreed that the highest content of CRF is found in the stalk-median eminence complex of the rat. The presence of a hypothalamic SRIF-neuronal system has also been detected in the rat (Dierickx and Vandesande 1979, Krisch 1979, Kawano et al. 1982, Ibata et al. 1983, Vincent et al. 1985), in human (D sy and Pelletier 1977) and in primates (Filby and Gross 1983).

During the last two decades, numerous studies have shown that neurons synthesizing and releasing these peptides form an integral component of the central nervous system. To date it is well established that these nerve cells being

termed "peptidergic neurons", are distributed also in several extrahypothalamic areas as well as in the peripheral nervous system and other organs or tissues of the organism.

In extrahypothalamic brain areas, TRH was demonstrated in the pineal body (Jackson and Reichlin 1974), in the thalamus, forebrain and brainstem (Kordon et al. 1977), in the mesencephalon, the lower brainstem and the spinal cord (Hökfelt et al. 1975) and in the circumventricular ependymal organs of the rat (Kizer et al. 1976). Similarly, LHRH has also been found to be present in several extrahypothalamic locations: in the medial septal area, the anterior hippocampus, the olfactory bulb and the piriform cortex in the rat (Sétáló et al. 1976, Krisch 1980, Joseph et al. 1981, Merchenthaler et al. 1984) and in the mouse (Silverman et al. 1979). CRF-containing neurons have been demonstrated throughout the entire limbic system (septum, hippocampus, amygdala, olfactory bulb), in the cortex, in the mesencephalon (periaqueductal gray, dorsal raphe nucleus) and in the spinal cord (Olschowska et al. 1982, Fischman and Moldow 1982, Vigh et al. 1984). SRIF-containing neurons have also been detected in several extrahypothalamic regions (Krisch 1979). A detailed analysis of the SRIF system in the spinal cord of the rat has been reported by Krukoff et al. (1986).

The presence of releasing and inhibiting hormones has also been shown in organs outside the central nervous system of vertebrates and even in invertebrates. TRH was demonstrated in the retina (Schaeffer et al. 1977), in the gastrointestinal tract and pancreas of the rat (Morley et al. 1977), in the skin of the frog (Jackson and Reichlin 1977), in the brain of the lamprey and in the head region of the protochordate amphioxus (Jackson and Reichlin 1974). Similarly, SRIF was also found in the gastrointestinal tract and in the endocrine part of the pancreas of the human (Chaivialle et al. 1980). Additional examples could be presented considering other RHs and IHs; since our work is restricted to the intracerebral localization of these neuropeptides, further discussion of the extracerebral appearance of these hormones has been omitted.

The localization of the growth hormone-releasing hormone (GHRH)-containing neuronal structures has also been investigated in mammals. Since antisera generated against rat GHRH and/or human GHRH have not shown cross-reactivity with the GHRH of birds, we have not investigated this system; therefore, references concerning the GHRH system have not been listed. Furthermore, we have to omit the discussion of all the other purported RH- and IH systems, such as the melanocyte-stimulating hormone and prolactin-releasing and inhibiting hormones (MSH-RH and IH; PRL-RH and IH). These RHs and IHs have not been chemically identified yet; thus, no antisera generated against these peptides are available for analysis of their location and ontogenesis.

Considerably less data are available in the literature concerning the localization of RHs and IHs in the central nervous system of birds; however, the hypothalamo-hypophyseal system of birds has attained a high degree of specialization not inferior to that of mammals. The identification of hypothalamic regions that take part in the regulation of pituitary function is an essential prerequisite for the environmental control of endocrine functions also in birds.

The hypothalamic control of the pituitary-gonadal axis has been documented in birds, too. Based on studies using various deafferentation and lesioning interventions in the brain, it has been indicated that both the preoptic region and the tuberal hypothalamic area are important in the regulation of the gonadotropin secretion in the duck (Assenmacher 1958, Novikov and Roudneva 1964), quail (Wada 1974, Oliver and Baylé 1976, Davies and Follett 1980), pigeon (Bouille and Bayle 1973) and turkey (Opel 1979). The presence of immunoreactive LHRH-fibres has been reported for the first time in the median eminence of the duck (Calas et al. 1973). Subsequently, the LHRH-immunoreactive neuron system has been demonstrated in several avian species, in the mallard (McNeill et al. 1976, Bons et al. 1978), domestic fowl (Sterling and Sharp 1982) and the Japanese quail (Oksche 1978, Hattori et al. 1980, Mikami 1986). These investigations were carried out using antisera generated against synthetic mammalian Arg₈-LHRH. The hypothalamic location of the avian LHRH system, reported by the above-mentioned authors, appeared to be fairly similar to those observed in mammals; although, the extrahypothalamic LHRH-IR system seemed to be less extensive in birds than in mammals.

The location of the hypothalamic CRF-containing neural system in birds has been studied with the aid of several physiological experiments suggesting the predominance of the rostral hypothalamus in CRF-production. Electrolytic lesions placed in the preoptic area of the pigeon caused significant decrease in the basal levels of plasma corticosterone (Bouille and Bayle 1973). Bioassay studies of CRF activity in various hypothalamic regions of the pigeon have suggested that only the ME and the anterior periventricular region contain significant amounts of activity (Péczy 1969). Other studies (Abel et al. 1975) have provided evidence that the hypothalamic paraventricular nucleus (PVN) of the Pekin duck is directly involved in the regulation of ACTH secretion. Moreover, a significant corticoid uptake of the parvocellular elements of PVN has also been shown. On the basis of *in vitro* studies, Péczy (1969) suggested that the site of the CRF production of the pigeon brain might be in the supraoptic area. Other studies indicated that the corticotropic area of the avian brain is located in the medio-basal hypothalamus (Frankel et al. 1976). Carsia et al. (1984) demonstrated that synthetic CRF stimulates ACTH secretion from the domestic fowl pituitary. The CRF neural system has also been investigated

by means of immunocytochemistry in several avian species using antisera generated in rabbits against synthetic ovine 41-CRF. In all studies, the rostral hypothalamus and mainly the paraventricular nucleus appeared to be the major hypothalamic location of CRF-IR perikarya (chicken: Józsa et al. 1984, pigeon: Péczely and Antoni 1984, Fellmann et al. 1984, quail: Mikami 1984, duck: Blähser 1984). The extrahypothalamic distribution of CRF-IR cells was reported to be considerably more circumscribed in birds than in mammals. In contrast to mammals, no CRF-IR perikarya were observed in telencephalic areas of birds homologous to the mammalian neocortex.

The phylogenetic distribution of TRH has been determined by sensitive radioimmunoassay in hypothalamic extracts of man, pig, hamster, frog, snake, salmon and chicken (Jackson and Reichlin 1974). These findings proved that TRH is distributed throughout the vertebrate kingdom indicating that it has an ancient lineage. The authors reported that the distribution of TRH in the chicken hypothalamus parallels that in the rat. Smaller concentrations of TRH were found in the extrahypothalamic brain tissue of both the rat and the chicken, with the highest concentration in the olfactory lobe. The localization of TRH-IR neuronal elements has so far been described in the brain of the chicken embryo (Thommes et al. 1985). These very sparse data do not allow the estimation of the extrahypothalamic localization of TRH in birds, compared to the wide distribution of this peptide in the central nervous system of different mammalian species.

The SRIF-neural system appears to be the most thoroughly studied IH system in birds. A widespread distribution of SRIF-containing neurons in different parts of the central nervous system has been established in different avian species by means of immunocytochemical methods. Dubois (1976) demonstrated SRIF-immunoreactive (SRIF-IR) axons in the ME of the domestic fowl, and Calas et al. (1975) described SRIF-IR material in perikarya near the vascular organ of the lamina terminalis and in the nerve terminals in the median eminence of the domestic mallard. Blähser (1978) found SRIF-IR cells in the supraoptic and paraventricular nuclei of the hypothalamus and in the preoptic area of the domestic mallard. SRIF-IR cells, fibres and terminals were mapped in the brain of the grass parakeet (*Melapsitaccus undulatus*) in a very detailed immunohistochemical study by Takatsuki et al. (1981) and Shiosaka et al. (1981). Large numbers of SRIF-IR neurons were demonstrated in the following hypothalamic areas: medial hypothalamic nucleus, posterolateral hypothalamus and mammillary nucleus. Immunoreactive fibres were also traced running towards the median eminence. Widespread distribution of SRIF-IR nerve cells was shown in extrahypothalamic regions. In the telencephalon the parolfactory lobe, the hippocampus and the palaeostriatum contained numerous SRIF-IR perikarya (Takatsuki et al. 1981). The interpeduncular nucleus and central gray of the mesencephalon, the tectum opticum and the spinal cord were shown to

contain a great concentration of SRIF-IR cells as well (Shiosaka et al. 1981).

After this brief survey of the literature it turns out evidently that considerably less information is available concerning the neuroendocrine systems of birds than of the different mammalian species. The localization of different RH- and IH-containing neurons in the avian neural tissue is potentially of great interest because of the well-characterized environmental control of endocrine functions in birds. Moreover, a number of well-defined hypothalamic and extrahypothalamic cell groups in the avian brain are implicated in the regulation of autonomic and vegetative functions such as eating, drinking and reproductive behaviour. It is necessary to characterize the distribution of RHs and IHs in these areas to obtain a better understanding of the functions of these neuropeptides in the regulation of autonomic functions and behaviour. These facts prompted us to perform systematic investigations on the localization and projections of the different RH- and IH neuron systems in the avian brain.

Similarities and Differences in the Ontogenesis of the Releasing and Inhibiting Hormone Systems in Mammals and Birds

Relatively few studies have been carried out to monitor the ontogenetic development of the different releasing and inhibiting hormone-containing neural elements both in mammals and birds. There is a wide variation in the duration of pregnancy (the time of ontogenetic development) in mammals (e.g. 3 weeks in the rat and nearly an entire year in the donkey), while the incubation time of the eggs of different avian species is significantly shorter. Therefore, it seems to be difficult to make direct comparisons in the sequence of appearance of the different RH- and IH neuron systems between mammals and birds. The trend of development of these systems in different parts of the brain, however, might be compared with certain restrictions.

A few representative data on the onset of production of the RHs and IHs in different mammalian species are summarized in *Table II*. As it is shown, the onset of LHRH production in the rat was found to occur in a period ranging from embryonic day 15 to the 1st postnatal day according to the data of different authors. LHRH-expressing neurons were detected in the olfactory bulb of the rat on embryonic day 15 (Jennes 1989). The author points out, however, that the LHRH-containing neuronal system in the brain develops rapidly during the subsequent days. These latter observations are in accordance with previous findings.

TRH appears in the central nervous system of the rat on the last days of gestation. The onset of CRF production appears in the same species between day 15–17. GHRH is found on day 20, while SRIF is detectable in the fetal rat

Table II

Onset of production of the releasing and inhibiting hormones in different mammalian species

Releasing and inhibiting hormone	Species	Day of onset of production	References
LHRH	rat	1st P	Ibata et al. (1981)
	rat	1st P	Araki et al. (1975)
	rat	22nd E	Barnea et al. (1977)
	rat	20th E	Daikoku et al. (1978)
	rat	19th E	Watanabe (1980)
	rat	18th E	Sétáló et al. (1978a)
	rat	17th E	Horváth et al. (1982)
	rat	16.5th E	Daikoku et al. (1984b)
	rat	15th E	Jennes (1989)
	mouse	11.5th E	Schwanzel-Fukuda and Pfaff (1989)
	guinea pig	28th E	Wray et al. (1989a, b)
	human	13th Ew	Schwanzel-Fukuda et al. (1981)
			Bugnon et al. (1977a)
CRF	rat	17th E	Grino et al. (1989)
	rat	16th E	Bugnon et al. (1982c)
	rat	15th E	Rundle and Funder (1988)
	human	16th Ew	Bugnon et al. (1982a)
TRH	rat	22nd E	Barnea et al. (1977)
	rat	19th E	Schaeffer and Brownstein (1980)
	mouse	12th E	Faivre-Bauman et al. (1978, 1980)
GHRH	rat	20th E	Degennaro et al. (1986)
	human	18th Ew	Bresson et al. (1984)
SRIF	rat	1st 1P	Horváth et al. (1982)
	rat	22nd E	Ho (1977)
	rat	14th E	Shiosaka et al. (1982)
	rat	12.5th E	Daikoku et al. (1983)
	mouse	19th E	Gross and Longer (1979)
	human	16th Ew	Bugnon et al. (1977b)
	human	16th Ew	Chaivialle et al. (1980)

E=embryonic days; P=postnatal days; Ew=embryonic week

from day 12.5 on. Data obtained on rats might have special importance from the point of view of comparison between birds and mammals. The duration of embryonic development is nearly the same (21–22 days) in the rat, the most widely used experimental mammalian species, and in the chicken, the most commonly used laboratory bird species; this comparison will be discussed later.

Similar to the studies performed on adults, considerably less data are available dealing with the ontogenesis of the RH- and IH neuron systems in birds compared to mammals. Blähser and Heinrichs (1982) compared the ontogenetic developmental pattern of three most commonly used experimental avian species: the domestic mallard, the domestic fowl and the Japanese quail. In the brain, LHRH-immunoreactive material was discernible in the chicken and quail on embryonic days 14–15 and in the mallard on day 20. The LHRH neurons were detected first in the septo-preoptic area. Contrary to these data, another group reported the appearance of LHRH at a quite early stage of development, on day 5.5 in the developing chicken brain (Woods et al. 1985). Similar to mammals, migration of LHRH cells from the olfactory placode has also been reported in the chicken embryo (Murakami et al. 1991, Akutsu et al. 1992). Regarding the fact that LHRH neurons migrate, the differences in data reporting the initial appearance of LHRH might be interpreted as if the authors described the appearance of LHRH cells after completing their migration (Blähser and Heinrichs 1982) or right after the first migratory cells appeared in the developing forebrain (Woods et al. 1985).

No data have been found indicating the onset of CRF production in the avian embryos. At present, only one report is available on the appearance and development of TRH-producing neurons in chicken embryo (Thommes et al. 1985): the first day of appearance was detected on day 4.5. Since no data are available indicating migratory activity of TRH neurons similar to the LHRH neurons, the significance of the appearance of TRH-immunoreactive neurons at this early stage of development remains an open question.

SRIF-containing perikarya appeared first in the chicken on day 7 of incubation, in the quail on day 11, and in the mallard on day 15 (Blähser and Heinrichs 1982). SRIF-immunoreactive cells were visible first in the brainstem, in the paraventricular nucleus, but later on several other extrahypothalamic areas (olfactory bulb, mesencephalon, pons) also contained SRIF-IR material.

Chapter 2. MATERIALS AND METHODS

Animals and General Experimental Conditions

Animals

The easily accessible domestic birds, the chicken and the mallard, were used, mainly for practical reasons, in our studies. Since antisera raised against synthetic mammalian RHs or IHs have shown cross-reactivity with the corresponding RHs or IHs of these domestic birds, there was no reason to use other bird species. It would be interesting, of course, to make interavian comparative studies, but our scope was a more general, principal comparison between birds and mammals.

The ontogenetic studies were performed on the same avian species used also for mapping the localization (LHRH, CRF and SRIF in the chicken; TRH in the mallard). It should be stressed here that the incubation period comprises 21 days in the chicken and 28 days in the mallard. This difference should always be taken into account when comparing the data of appearance of the RHs and IHs in the different avian species used in our studies.

Adult New Hampshire and Plymouth hens (6–24 months of age) were used. LHRH experiments were carried out on hens reared in outdoor aviaries under natural day-night photoperiods. CRF and SRIF investigations were performed on birds housed in individual cages (1 × 1 × 1 m) with a 12:12 light-dark schedule. Hens were fed with natural corn and oats. Water and food were available *ad libitum*. For TRH studies adult Pekin ducks of both sexes were used; they were fed with dry chicken food and had free access to water.

For the ontogenetic studies, the fresh fertilized eggs of New Hampshire chickens and Pekin ducks were artificially incubated in a humid chamber (38.1 °C). Embryos were collected at 24 h intervals from day 10 to day 21 of incubation (day of hatching) of the chicken, and from day 10 to day 28 (day of hatching) of the mallard. Subsequent studies were performed on 2-, 7-, 18- and 30-day old chickens, and on 1- to 7-day-old ducks after hatching. Each age group of embryos and young birds consisted of at least five animals.

Colchicine Application

Using immunohistochemical techniques, immunoreactive nerve fibres could be visualized in untreated animals; however, special experimental procedures were necessary to observe the cell bodies as well. Colchicine, a mitosis inhibitor known to arrest the axonal transport of neuropeptides, was administered intracerebroventricularly. By inhibiting the intra-axonal transport, the low concentration of the hormones present in the perikaryon increased and the perikaryon could also be visualized.

Hens, weighing 2000–3000 g, were anaesthetized with hexobarbital (40 mg/kg) and received colchicine (80–100 µg/10 µl physiological saline) stereotactically injected into the lateral ventricle. Ducks were treated with the same dose of colchicine injected freehand into the lateral ventricle under X-ray control. The animals were allowed to survive for 48 hours, thereafter the brains were processed for immunocytochemistry.

Fixation and Section Preparation

Animals were perfused via the carotid artery with 200 ml ice-cold phosphate-buffered saline (PBS, 0.01 mol/l, pH 7.4) followed by 400 ml of picric acid–formaldehyde solution (Stefanini et al. 1967). After perfusion, the brains were carefully removed from the skull and submerged in the same fixative for 3 days at 4 °C, then washed in PBS for 8–10 h. Following dehydration in graded alcohols (70, 90, 96, 100%), brains were embedded in paraffin (LHRH, CRF and SRIF studies). In the case of TRH studies, the brains were postfixed in the same fixative for 1 day and rinsed overnight in PBS containing 20% sucrose. Thereafter, the tissue was frozen on dry ice and cut in a Leitz-cryostat. Serial coronal and sagittal 20 µm sections were prepared and mounted on gelatin-coated slides. The sections were treated with 0.2% Triton X-100 solution to facilitate the penetration of antibodies.

At early embryonic ages, after decapitation, the brains of the embryos were fixed by immersion technique. Duck embryos, older than 14 days, and newly hatched chickens and ducklings, were perfused via the left cardiac ventricle. The brains of embryos younger than 12 days (chicken) and younger than 14 days (ducks) were sectioned together with the skull. For perfusion, the same fixative was used as in the adults.

Immunocytochemistry

Antisera

1) *Anti-LHRH* (rabbit) No. 16. Optimal dilution: 1:30,000.

The antiserum was raised in our department by J. Horváth. Synthetic p-glutamyl-LHRH (Calbiochem) was conjugated to bovine serum albumin (BSA) as an antigen by glutaraldehyde, according to the method of Reichlin et al. (1968).

2) *Anti-CRF* (rabbit) No. SV 22. Optimal dilution: 1:4000.

The antiserum was made by coupling synthetic 41-residue ovine CRF-41 to BSA with glutaraldehyde in our department; the antiserum recognizes mainly the C-terminal portion of the CRF-41 chain. The antiserum was produced and characterized by Vigh et al. (1982).

3) *Anti-TRH* (rabbit) No. RTH 44. Optimal dilution: 1:10,000.

The antiserum was raised in rabbits against L-pyroglutamyl-histidyl-proline (free acid; synthesized at Gedeon Richter Chemical Works, Budapest) conjugated to fatty-acid-free BSA at its C-terminal carboxyl group by the mixed anhydride technique (Erlanger et al. 1957) with minor modifications by V. Csernus (in our department).

4) *Anti-somatostatin* No. 101 (gift from T. Görcs). Working dilution: 1:800; No. 902 (gift from P. Petrusz). Working dilution: 1:4000.

5) *Sheep (or swine) anti-rabbit gamma globulin (a-RGG)*.

1:50: raised in our laboratory (in LHRH, CRF studies);

1:80: DAKO, Copenhagen (in TRH study);

1:600: Arnel, New York, NY (in SRIF study).

6) *PAP* (soluble complex of horseradish peroxidase-rabbit anti-horseradish peroxidase).

1:200: Humán Oltóanyagtermelő és Kutatóintézet, Budapest (in LHRH, CRF studies);

1:80: DAKO, Copenhagen (in TRH study);

1:600: Arnel, New York, NY (in SRIF study).

Immunostaining

The immunocytochemical staining was performed according to the unlabelled peroxidase-antiperoxidase (PAP) technique of Sternberger et al. (1970).

The sections were first exposed to 2% normal sheep serum (NSS) or 10% normal swine serum for 30 min, at room temperature. The tissues were subsequently incubated with 1) the primary antiserum for 48 h at 4 °C, 2) the second antiserum (a-RGG) for 2 h at room temperature and 3) PAP, 20 min, at room temperature, respectively. All reagents were dissolved in PBS containing 2%

normal sheep (or swine) serum and 0.02% sodium azide, except for the DAB solution from which sodium azide was omitted. The final reaction was developed in a solution containing 0.025% 3,3'-diaminobenzidine-tetrahydrochloride (DAB) (Sigma: in TRH; Aldrich, Strasbourg, France: in CRF and LHRH studies) in the peroxidase reaction and 0.05% or 0.003% H_2O_2 (in TRH, the latter in CRF and LHRH studies) dissolved in TRIS buffer. Each incubation was followed by 10 min wash in PBS.

The specificity of the staining was controlled by 1) omitting either the specific antiserum, or the sheep antirabbit IgG, or the PAP complex; 2) replacing the specific antiserum with rabbit preimmune serum; 3) using increasing dilutions of the primary antiserum resulting in a gradual decrease and eventual disappearance of the staining and 4) staining with the primary antiserum after absorption with the appropriate antigen.

Immunization of rabbits with an antigen, results in a mixture of antibodies of variable affinity (polyclonal antibodies). The specificity of antibodies in an immunochemical system can be studied by antigens which compete with the tissue antigen for the binding sites of the antibody in question. Preabsorptions of the primary antisera used in our studies were carried out 24 h prior to use at 4°C, applying 10 µg/ml synthetic mammalian LHRH (Calbiochem), 7.5 µg/µl synthetic ovine CRF (Calbiochem) and 40–100 µM synthetic TRH (Gedeon Richter Chemical Works, Budapest), respectively. Preabsorption with the appropriate antigen per ml working dilution of the antiserum resulted in the abolition of the specific staining in birds, described below. Since the polyvalent antisera contain antibodies against different determinants of the antigen molecule, these antibodies may recognize the same amino acid sequences in structurally or functionally different molecules as well. Thus, theoretically it seems possible that our antisera may recognize unknown peptides, prohormons or anabolic fragments, etc. On the other hand, the chemical structure of some peptides has yet to be determined in birds (e.g. CRF). The present data should rather be interpreted as immunoreactive (IR) structures instead of peptide-containing structures.

The simultaneous immunohistochemical localization of CRF- and TRH-IR sites was accomplished by using a double labelling technique (Liposits et al. 1986). First, CRF-containing neurons were detected by the black, silver-gold intensified DAB-end product. Thereafter, TRH-containing neural elements were identified by the natural brown DAB-chromogen on the same tissue slide. The high contrast of the chromogens allows the distinctive separation of neural structures that express different immunoreactivities.

To avoid a possible major criticism concerning the lack of any statistical mathematical analysis, we have to stress that all our results are based on qualitative immunocytochemical data. This technique basically does not allow a real quantification. On the other hand, the goal of the present work was to

show either the presence or absence of RH- or IH-IR neuronal structures in a given area of the brain. In the second part (ontogenetic studies), the aim was again to detect the appearance of a given RH- or IH-IR substance at a certain stage of development in the investigated brain region. Since the question was "yes or no", and we did not draw any fundamental conclusion from the quantitative differences if visualized in regions of the central nervous system (e.g. different number, different staining intensity), a statistical mathematical analysis was not carried out under these conditions.

Chapter 3. INTRACEREBRAL LOCALIZATION OF THE RELEASING AND INHIBITING HORMONE-IMMUNOREACTIVE (RH-IR AND IH-IR) NEURON SYSTEMS IN BIRDS

Luteinizing Hormone-Releasing Hormone (LHRH)-IR System

It has been reported (Józsa and Mess 1982) that the greatest concentration of LHRH-IR perikarya is located in the septum, immediately rostral to the interventricular foramen (*Fig. 1A*). This cell group was observed in each age group, independent of the season. LHRH-containing cells were also present in the preoptic-periventricular area and along the wall of the third ventricle (*Fig. 1B*). A few LHRH neurons could also be detected in the olfactory bulb (*Fig. 1C*) and in the vicinity of the organum vasculosum of the lamina terminalis (OVLT). The cell groups in the preoptic-periventricular area, in the vicinity of the OVLT and in the septum were interconnected by scattered LHRH-IR perikarya located above and in front of the anterior commissure. However, it should be mentioned that LHRH-IR cells were found neither in the tuberal region of the hypothala-

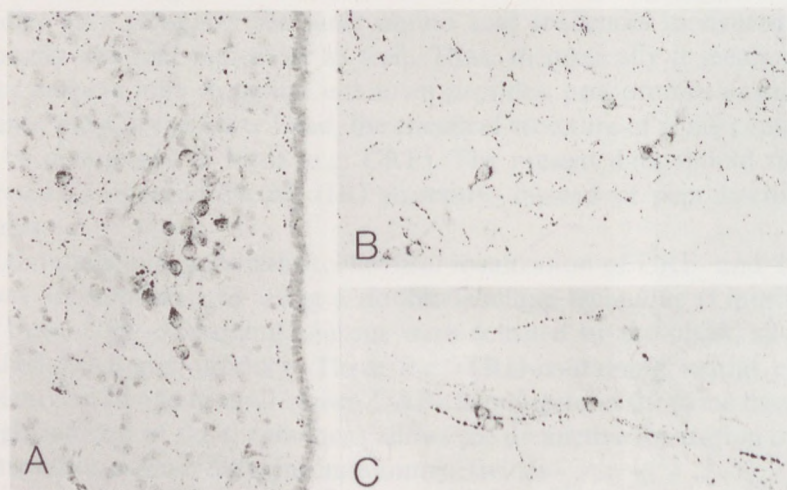


Fig. 1. Immunoreactive (IR) perikarya, immunostained with mLHRH-antiserum, in different brain regions of the domestic fowl. 10 μ m thick sagittal sections. Non-colchicine-treated animals. *A*) LH-RH-IR perikarya in the septum *B*) within the preoptic area and *C*) in the region of the olfactory bulb. $\times 200$

mus nor in the nucleus taeniae. This latter nucleus corresponded with the amygdaloid nucleus of mammals (Martinez-Vargas et al. 1976) that has been reported to contain numerous LHRH neurons.

In contrast to the perikarya, the majority of the LHRH-IR fibres and terminals was observed within the hypothalamus. The LHRH pathway, forming the

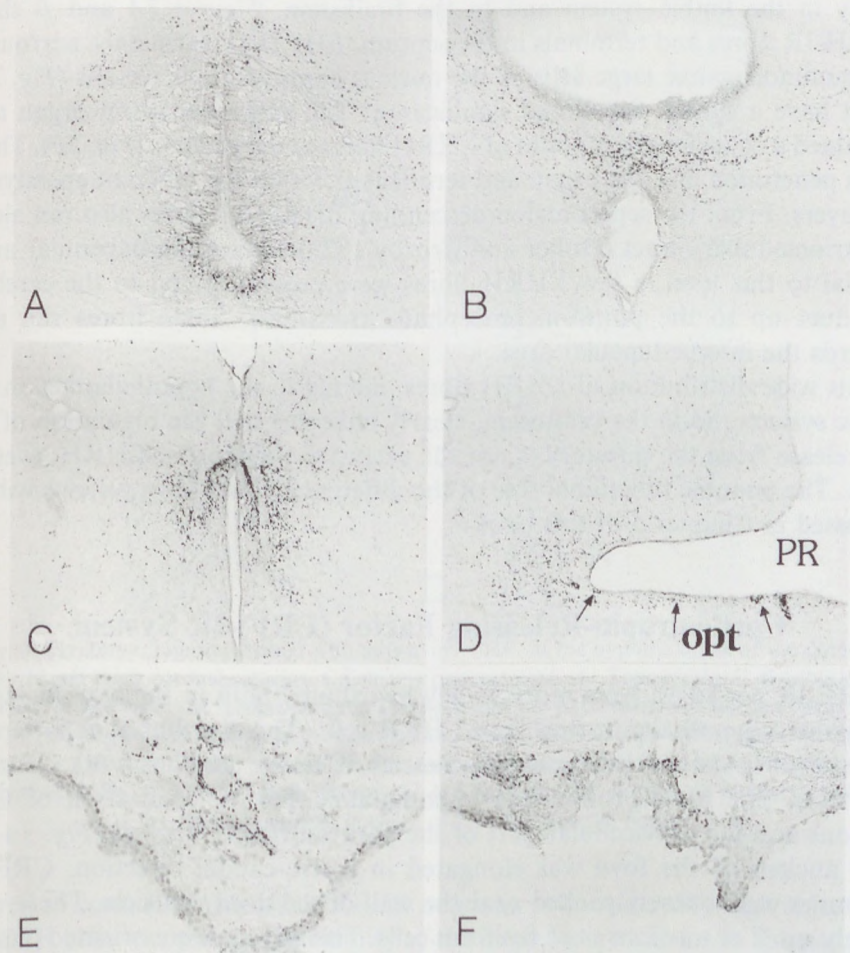


Fig. 2. Distribution of LHRH-IR fibres. 10 μ m coronal sections. Non-colchicine-treated animals. *A)* Dot-like LHRH fibres distributed around the bed nucleus of the pallial commissure. $\times 180$; *B)* Crossing LHRH-IR fibres in the septum. $\times 180$; *C)* Numerous LHRH-IR fibres in the preoptic region and in the organon vasculosum of the lamina terminalis (OVLT). $\times 180$; *D)* LHRH-IR fibres course towards the median eminence forming the preoptico-infundibular tract on both sides of the preoptic recess (PR) (arrows) and beneath the ependyma just dorsal to the optic chiasm (opt) $\times 110$; *E)* LHRH-IR nerve terminals around unstained cells of the nucleus commissuralis tectalis. $\times 280$; *F)* Immunoreactive nerve terminals in the subcommissural organ. $\times 280$

preoptico-infundibular tract, ran towards the median eminence (ME). This fibre system was located caudally from the preoptic region, under the ependyma of the third ventricle and just dorsal to the optic chiasm (*Fig. 2D*). Another LHRH-IR bundle, the so-called preoptico-terminal tract, courses towards the OVLT (*Fig. 2C*). Besides these two main pathways, LHRH-IR fibres and terminals were distributed also in different extrahypothalamic sites: predominantly in the limbic system and in the brainstem. *Figures 2A* and *B* show LHRH-IR fibres and terminals in the septum. LHRH-IR terminals, surrounding immunonegative large cells of the nucleus commissuralis tectalis (*Fig. 2E*) might have a special functional significance. The subcommissural organ also contained a considerable amount of LHRH fibres and terminals (*Fig. 2F*). These fibres penetrated into the organ and terminated within the different ependymal cell layers. From the septal region descending LHRH-IR fibres also ran along the striomedullary tract (Huber and Crosby 1929) toward the habenular area. Caudal to this level, a few LHRH fibres were present lateral to the cerebral aqueduct up to the ponto-mesencephalic transition. Some fibres ran also towards the interpeduncular area.

This wide distribution of LHRH fibres, mainly in the hypothalamus, in the limbic system and in the brainstem, clearly indicates that the regulation of the LH release from the pituitary is not the exclusive function of LHRH, even in birds. The possible functional role of the different LHRH fibre systems will be discussed in Chapter 5 of this book.

Corticotropin-Releasing Factor (CRF)-IR System

CRF-IR perikarya were more widely distributed both in the hypothalamus and in extrahypothalamic sites than LHRH-cells. The distributional pattern of this system in the chicken brain was described in our previous work (Józsa et al. 1984). The most pronounced hypothalamic site of localization of CRF neurons was the parvicellular part of the paraventricular nucleus (*Fig. 3A-B*). This nucleus of the fowl was elongated in rostro-caudal direction. CRF-IR perikarya were densely packed near the wall of the third ventricle. These were mainly small or medium sized fusiform cells. Their axons were oriented parallel to the wall of the third ventricle. Numerous CRF-IR cell bodies were also found in the preoptic area (*Fig. 3C-D*). These cells were arranged along the wall of the preoptic recess of the third ventricle and in the anterior preoptic nucleus. Besides these two main locations, scattered CRF-IR perikarya could be seen throughout the hypothalamus. A smaller group of CRF-IR cells was observed also in the mammillary region.

In extrahypothalamic brain areas, the dorsomedial and dorsolateral thalamic nuclei revealed strong CRF-immunoreactivity (*Fig. 4A-B*). These nuclei ap-

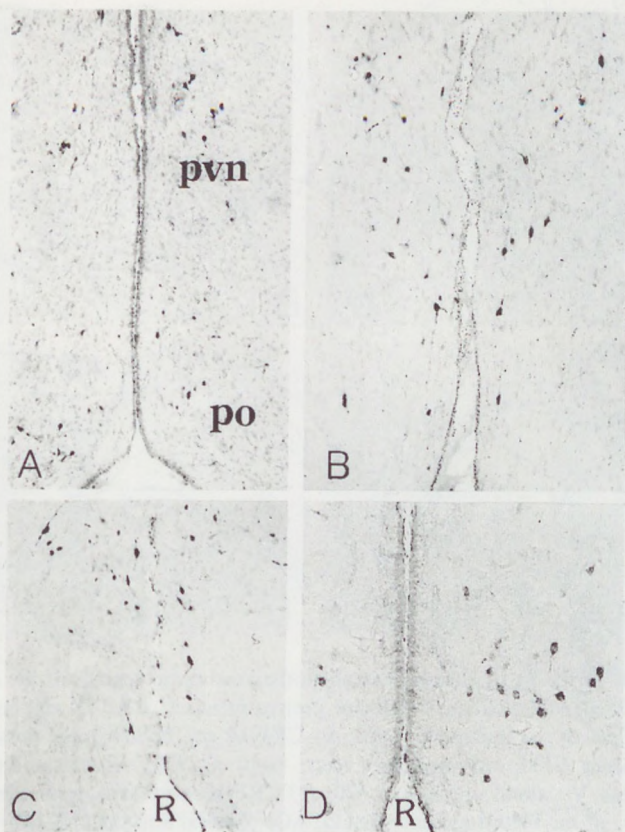


Fig. 3. Perikarya, immunostained with anti-ovine CRF, in the hypothalamus of the domestic fowl. 15 μ m thick coronal sections. Colchicine pretreatment. *A*) Topographical distribution of CRF-IR perikarya in the medial preoptic area (po) and the paraventricular nucleus (pvn). $\times 80$; *B*) CRF-IR perikarya in the periventricular and medial subdivisions of the paraventricular nucleus. $\times 110$; *C*) CRF-IR perikarya in the periventricular preoptic area along the wall of the third ventricle $\times 110$ R: preoptic recess; *D*) A cluster of CRF-containing perikarya in the medial preoptic nucleus. $\times 160$; R: preoptic recess

peared to contain multipolar CRF-IR neurons. A considerably high amount of CRF-IR cells was seen in the vicinity of the lateral ventricle, i.e. in the parolfactory lobe and in the region of the accumbens nucleus (*Fig. 4C*). The brainstem also contained several CRF-IR perikarya located in the mesencephalon around the ventral portion of the cerebral aqueduct (*Fig. 4D*), and in the region of the oculomotor nucleus.

High numbers of CRF-IR fibres and terminals were found in the external zone of the anterior ME and only some fibres were located in the internal zone. Several CRF-IR fibres were observed entering the OVLT as well.

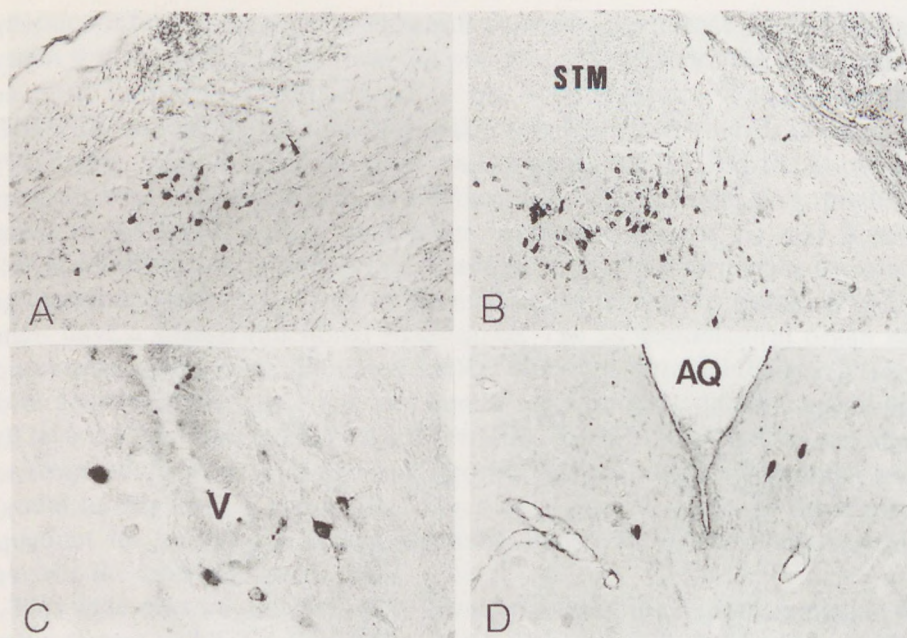


Fig. 4. CRF-IR perikarya in different extrahypothalamic brain regions of the domestic fowl.

15 μ m thick coronal sections. Colchicine pretreatment. *A)* CRF-IR cell bodies in the dorsolateral nucleus of the thalamus. $\times 110$; *B)* CRF-IR cell bodies in the dorsomedial nucleus of the thalamus. STM: striomedullary tract. $\times 110$; *C)* CRF-IR cells and fibres in the accumbens nucleus. V: lateral ventricle. $\times 320$; *D)* CRF-IR perikarya in the periventricular gray of the rostral mesencephalon. AQ: cerebral aqueduct. $\times 200$

Thyrotropin-Releasing Hormone (TRH)-IR System

The TRH system is the most widely distributed RH system. Numerous TRH-IR neuronal elements were detected in the hypothalamus and also in several extrahypothalamic brain regions. Our results demonstrated for the first time the distribution of the TRH-IR system in the brain of the mallard (Józsa et al. 1988). The location of TRH-IR perikarya, fibres and terminals is illustrated in *Fig. 5A–F*. The schematic drawings of frontal sections of avian brain are based on the atlas of Karten and Hodós (1967).

TRH-IR perikarya were highly concentrated within the hypothalamus. The parvocellular portion of the paraventricular nucleus contained the largest group of TRH-IR cells (*Fig. 6A*). No TRH-IR material was detectable in the magnocellular neurons either in the paraventricular or in the supraoptic nucleus. A large number of TRH-IR perikarya was located in the periventricular, medial and lateral subdivisions of the paraventricular nucleus. The parvocellular labelled cells exhibited a round or fusiform shape. The axonal

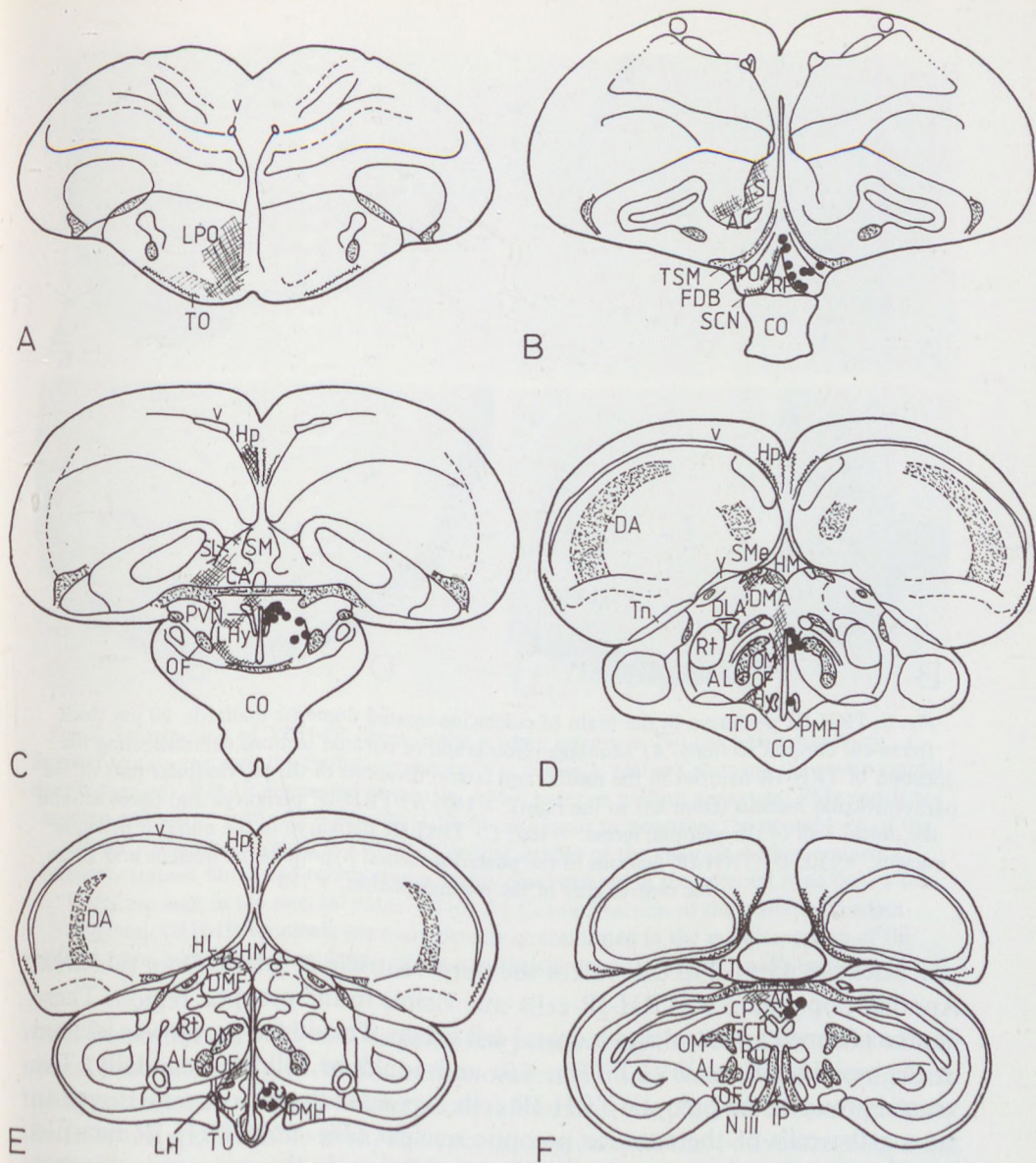


Fig. 5. Schematic representation of TRH-IR perikarya (dots) and fibres (shaded areas) in coronal sections at six representative levels of the brain of the domestic mallard. The drawings are based on the atlas of Karten and Hodós (1967) AC: nucleus accumbens; AL: ansa lenticularis; AQ: cerebral aqueduct; CA: anterior commissure; CO: optic chiasm; CT: tectal commissure; DA: tractus archistriatalis dorsalis; LA: nucleus dorsolateralis anterior thalami; DLA: nucleus dorsolateralis anterior thalami; DMA: nucleus dorsomedialis anterior thalami; DMP: nucleus dorsomedialis posterior thalami; FDB: diagonal fascicle of Broca; GCT: central gray of the mesencephalon; HL: nucleus habenularis lateralis; HM: nucleus habenularis medialis; Hp: hippocampus; IP: interpenduncular nucleus; LHy: nucleus lateralis hypothalami; LPO: parolfactory lobe; N III: oculomotor nerve; OM: occipitomesencephalic tract; POA: nucleus preopticus anterior; PMH: nucleus medialis hypothalami posterioris; PVN: paraventricular nucleus; QF: quintofrontal tract; RP: nucleus reticularis pontis caudalis; Rt: nucleus rotundus; Ru: red nucleus; SCN: suprachiasmatic nucleus; SL: nucleus septalis lateralis; SM: nucleus septalis medialis; SMe: medullary stria; T: triangular nucleus; Tn: nucleus taeniae; TO: olfactory tubercle; TrO: optic tract; TSM: septomesencephalic tract; TU: tuberal nucleus; V: ventricle

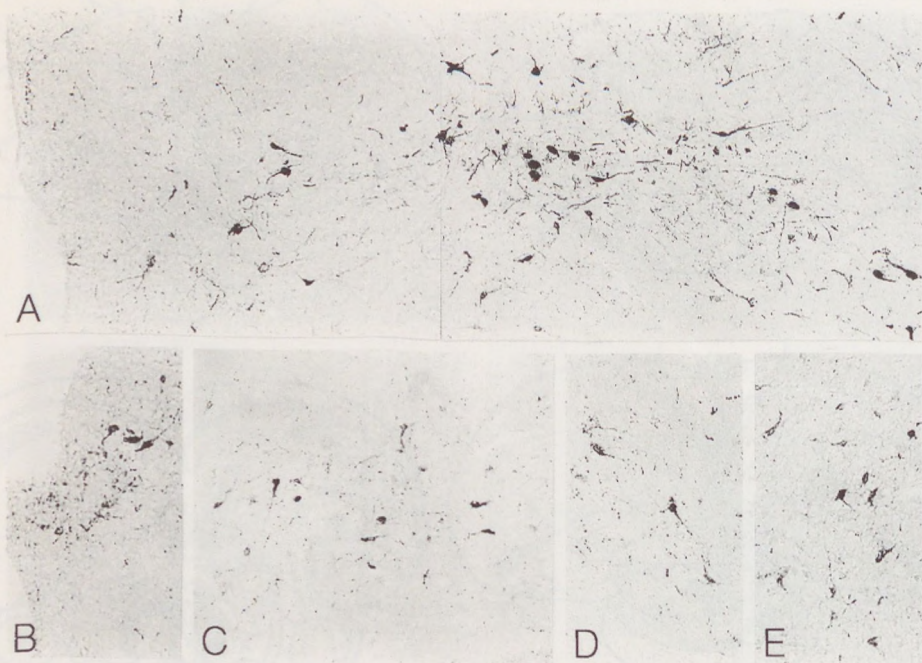


Fig. 6. TRH-IR perikarya in the brain of colchicine-treated domestic mallards. 20 μ m thick freeze-cut coronal sections. *A)* Montage photograph of coronal sections demonstrating the location of TRH-IR neurons in the medial and lateral divisions of the parvicellular part of the paraventricular nucleus (from left to the right). $\times 160$; *B)* TRH-IR perikarya and fibres around the dorsal wall of the preoptic recess. $\times 160$; *C)* TRH-IR perikarya in the anterior preoptic nucleus. $\times 110$; *D)* TRH-IR neurons in the posterior medial hypothalamic nucleus and *E)* in the central gray of the mesencephalon. $\times 110$

processes ran parallel to the wall of the third ventricle or lateral from this area. Another population of TRH-IR cells was visible in the preoptic region. These neurons formed a ventral and a dorsal cell group. The dorsal group was located in the vicinity of the OVLT (*Fig. 6B*) and could be followed caudally. The ventral group of the preoptic TRH-IR cells expanded in medio-lateral direction among the cells of the anterior preoptic nucleus (*Fig. 6C*). TRH-IR neurons were located also along the diagonal band of Broca, in the periventricular area around the wall of the preoptic recess, in the suprachiasmatic nucleus, in the posterior medial hypothalamic nucleus and in the lateral hypothalamic area (*Fig. 6D*). The most prominent extrahypothalamic area, where a cluster of TRH-IR cells had been detected, was the central gray of the rostral mesencephalon (*Fig. 6E*).

The densest accumulation of TRH-IR fibres and terminals was seen in the external zone of the anterior and posterior divisions of the ME in the vicinity of portal capillaries (*Fig. 7C, D*). A few TRH-IR fibres ran also in the internal

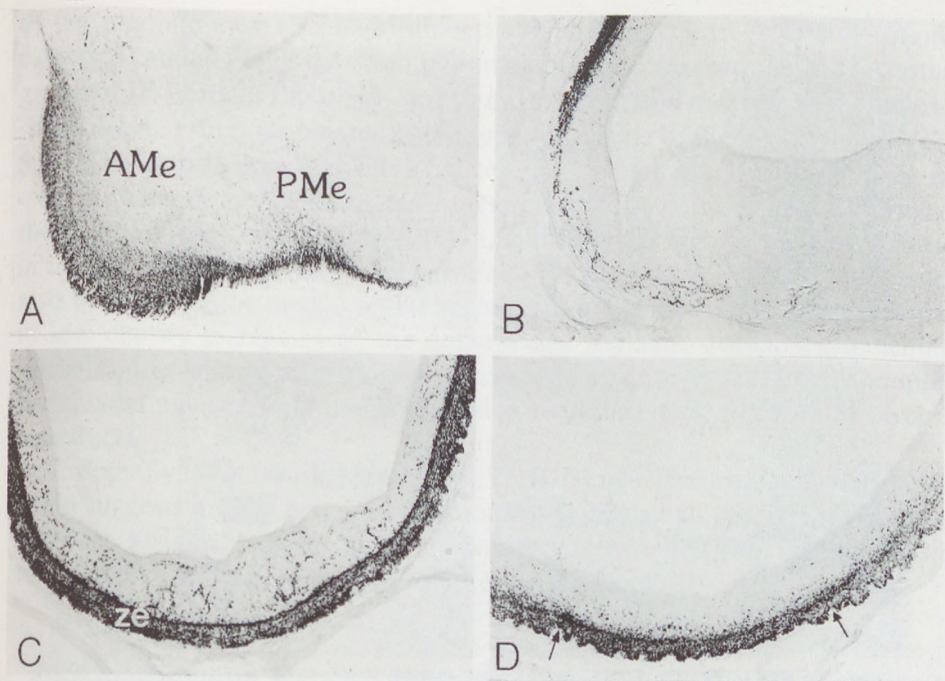


Fig. 7. Distribution of TRH-IR fibres in the median eminence. No-colchicine treatment. *A)* Sagittal section through the median eminence of a domestic mallard showing the rostro-caudal distribution of TRH-IR fibres and terminals. AMe: anterior median eminence; PMe: posterior median eminence. $\times 50$; *B)* A few TRH-IR fibres appear to penetrate the neural lobe of the pituitary. Coronal section. $\times 110$; *C)* Coronal section of the anterior median eminence. Intensely stained fibres and terminals are highly concentrated in the external zone (ze); a few fibres are seen in the internal zone. $\times 110$; *D)* Coronal section of the posterior median eminence. TRH-IR terminals are more densely concentrated in the middle portion of the external zone. Note the accumulation of the terminals around the portal vessels (arrows). $\times 110$

zone. Some fibres could also be followed penetrating the rostral portion of the neural lobe (*Fig. 7B*). Strongly immunoreactive fibres were found throughout the hypothalamus with particular accumulations in the paraventricular nucleus, the lateral hypothalamic area, the tuberal region and the periventricular area along the wall of the third ventricle (*Fig. 8A, E*). Nerve processes of TRH-IR neurons located in the paraventricular nucleus, where the majority of TRH-IR perikarya was located, coursed in three main directions. The majority of these fibres coursed laterally and then turned in a ventral direction toward the laterobasal surface of the hypothalamus. Fibres, coursing from the lateral hypothalamus, joined this bundle and a final common pathway was formed toward the ME (*Fig. 8B, C*). The second and third bundles, emerging from the paraventricular nucleus, consisted of considerably less fibres and travelled both ventrally and dorsally along the wall of the third ventricle. TRH-IR perikarya

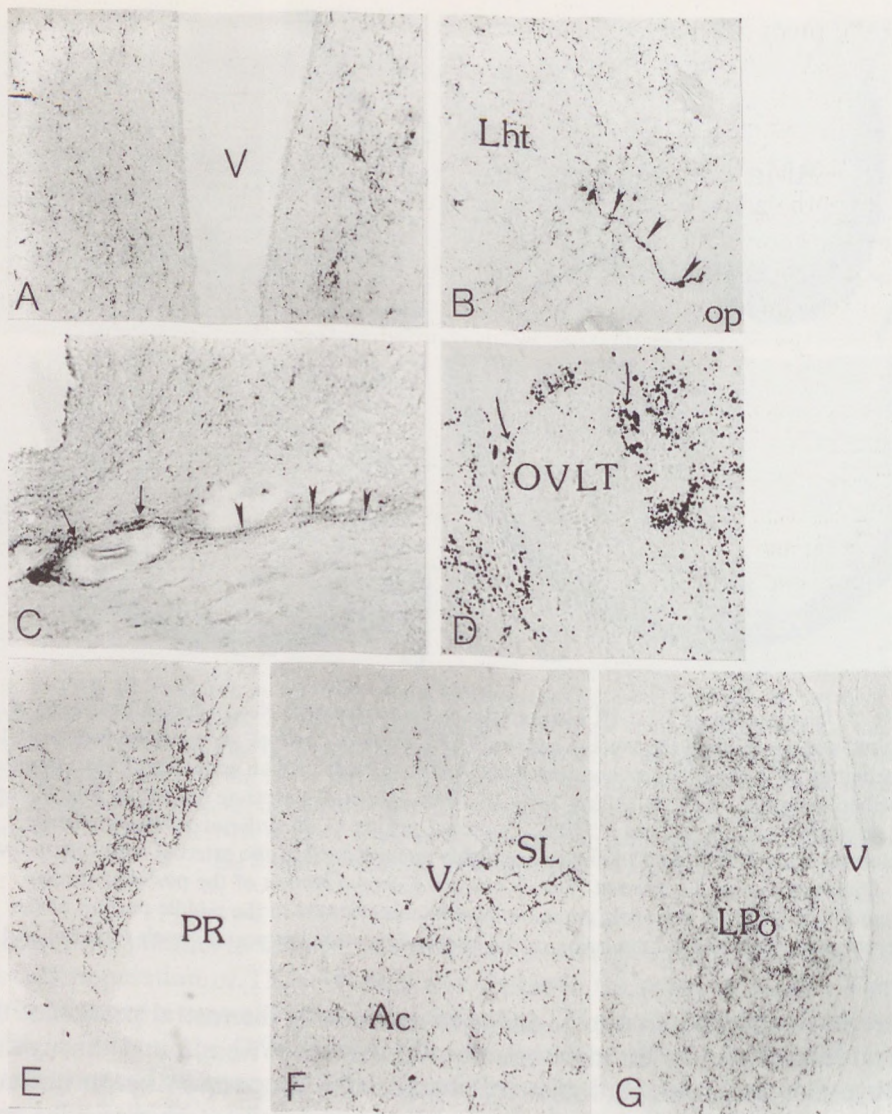


Fig. 8. Distribution of TRH-IR fibres in the brain of non-colchicine-treated domestic mallards. Coronal sections. *A)* Dense network of fibres located on both sides of the third ventricle at the level of the paraventricular nucleus. Fibres, running parallel to the surface, are frequently seen. V: ventricle $\times 160$; *B)* TRH-IR fibres in the lateral hypothalamic area (Lht). Note a heavily stained, long, varicose fibre running toward the basal surface (arrowheads); op: optic tract. $\times 160$; *C)* Immunoreactive axons (arrowheads) from a basal tract near the ventral surface of the basal hypothalamus as they run medially toward the median eminence. Note that fibres closely surround the external layer of the basal arteries (arrows). $\times 160$; *D)* TRH-IR dot-like structures (arrows) in the superficial layer of the organum vasculosum laminae terminalis (OVLT). $\times 280$; *E)* Fine fibre plexus around the wall of the preoptic recess (PR) in the preoptic-suprachiasmatic region. $\times 110$; *F)* Fibre network around the ventral edge of the lateral ventricle in the region of the accumbens nucleus (Ac) and the lateral septum (SL). V: lateral ventricle. $\times 110$; *G)* TRH-IR terminals in the parolfactory lobe (LPo); V: lateral ventricle. $\times 110$

of the preoptic area also had different axonal projections. A discrete bundle extended caudad along the basal hypothalamus and reached the ME. Another group of IR fibres of this origin was found superior and anterior to the anterior commissure. Fibres terminated in the superficial layer of the OVLT as well (*Fig. 8D*). The dorsal preoptic TRH-IR cell group had other characteristic extrahypothalamic projections, too. A very dense fibre network could be seen in the rostral portion of the lateral septum, in the accumbens nucleus (*Fig. 8F*) and in the subependymal layer of the parolfactory lobe (*Fig. 8G*). A few fibres could also be found in the medial septal nucleus. Furthermore, TRH fibres occurred in low density in regions of the dorsomedial thalamic nuclei, the habenular nuclei and in several parts of the mesencephalon (the central gray, the interpeduncular nucleus, the tectal areas and around the lateral edges of the cerebral aqueduct).

This nearly ubiquitous occurrence of TRH-IR neuronal elements in the avian brain suggests a more extended functional significance of this RH system than has been assigned exclusively to the pituitary release of TSH. This aspect will be discussed in detail in the last chapter of this book.

Somatotropin Release-Inhibiting Factor (SRIF)-IR System

As it has been mentioned, SRIF is the only IH that has been chemically identified yet, providing the possibility to generate a specific antiserum for conducting immunocytochemical studies.

Numerous immunoreactive perikarya were observed in the hypothalamus. A prominent group of labelled neurons were seen in the periventricular portion of the paraventricular nucleus (PVN), mostly in the anterior and middle portions of this nucleus (*Figs 9C and 10C*). The immunoreactive cells formed a long rostro-caudal chain within the PVN. From this peculiar formation, single cells or small neuronal clusters radiated toward the dorsolateral division of the PVN. A few SRIF-IR cell bodies were also visible in the supraoptic nucleus. Within the rostral hypothalamus, a few small and medium-sized cells were located in the lateral hypothalamus (*Fig. 10D*). Proceeding caudally, the number of SRIF-IR neurons decreased remarkably and no SRIF-IR cells were seen in the lateral hypothalamus at the level of the PVN. Several SRIF-IR cells could be detected in the nucleus medialis hypothalami posterior (PMH). A large group of SRIF-IR medium-sized cells was demonstrable particularly in its caudal part (PMHc) (*Fig. 9D*). Most of them occupied an area at some distance from the ventricle. Numerous cell bodies were also found in the infundibular nucleus (*Fig. 11A, B*). No SRIF-IR neurons occurred in the paraventricular organ. A large number of SRIF-IR perikarya were noted in the anterior preoptic region as well (*Figs 9A, B and 10A, B*).

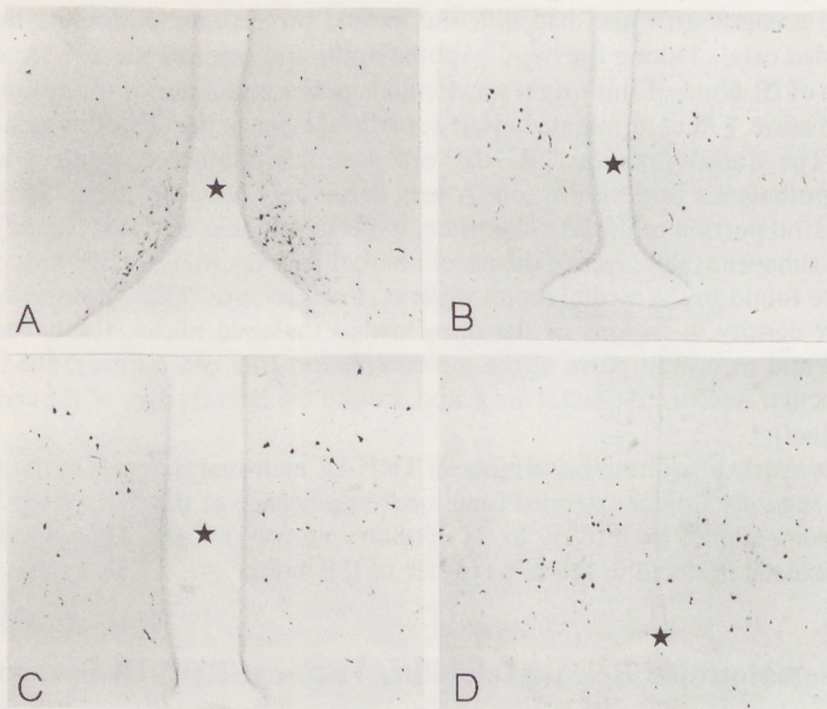


Fig. 9. Distribution of SRIF-IR neurons in the hypothalamus of the chicken. Coronal sections. $\times 50$. SRIF-IR perikarya located *A*) in the anterior preoptic region in close vicinity to the preoptic recess; *B*) in the medial preoptic nucleus; *C*) in the periventricular and medial subdivisions of the PVN and *D*) in the posterior hypothalamus. *third ventricle

SRIF-IR fibres were conspicuous in the rostro-dorsal portion of the hypothalamus and in the lamina terminalis. The densest accumulation of SRIF-IR fibres was seen in the ME (*Fig. 11C*). These fibres terminated in the external zone of both the anterior and the posterior divisions of ME. Some weakly immunoreactive fibres running in the inner portion of the external zone, appeared to join the hypothalamo-hypophyseal tract. No IR material could be detected in the pars distalis of the hypophysis. High density SRIF-IR fibres were seen in the ventral part of the PMH, in the lateral hypothalamus at the level of the mammillary body, and a moderate number in the rostral lateral hypothalamus. SRIF-IR fibres were concentrated also in areas where SRIF-IR neurons were detected.

A large number of SRIF-IR cells was found in the telencephalon, over the entire length of the hippocampus (*Fig. 12A, B*). The neurons appeared to spread laterally into the hyperstriatum accessorium and ventrally into the thin isthmus which connects the hippocampus and the septum. SRIF-IR fibres could also be traced to the septal area. No SRIF-IR perikarya, but only a moderate network of fibres, were found in the septum. This observation indicates that the sources

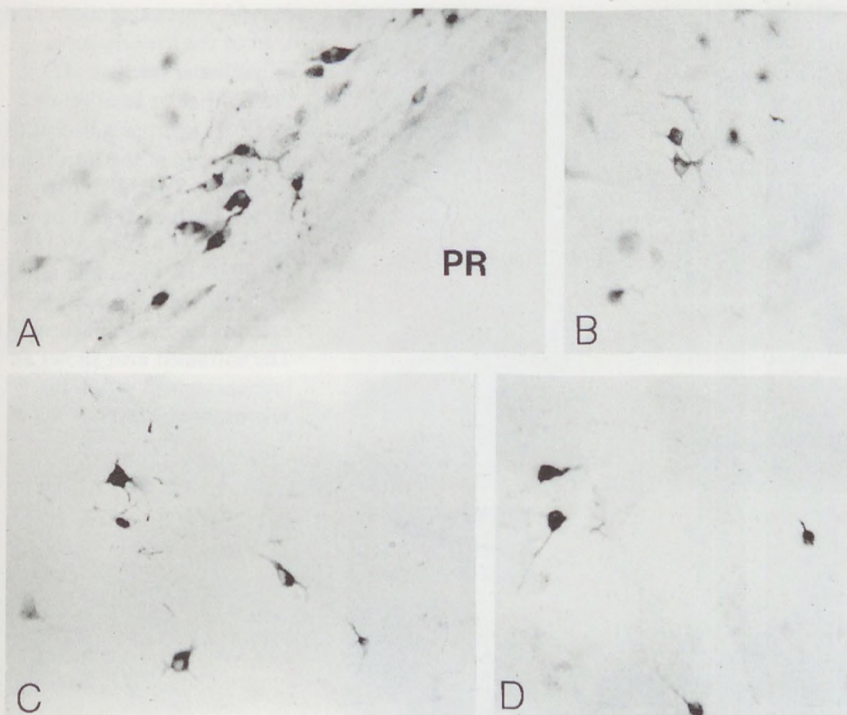


Fig. 10. Higher magnifications illustrating different SRIF-IR cell types in the *A*) anterior periventricular preoptic region; *B*) in the medial preoptic nucleus; *C*) in the medial subdivision of the paraventricular nucleus and *D*) in the rostral part of the lateral hypothalamic area. PR: preoptic recess. $\times 300$

of septal SRIF-fibres are extraseptal SRIF neurons. The rostral part of the olfactory lobe and tubercle, as well as the olfactory bulb contained several SRIF-IR neurons (*Fig. 13C*). These groups of cells were located close to the ventro-medial surface. Only a moderate number of SRIF-IR fibres was detected in the parolfactory lobe. Scattered SRIF-IR neurons were disseminated throughout the palaeostriatum augmentatum, however, no SRIF-positive neurons could be visualized in the archistriatum, neostriatum and hyperstriatum.

Large clusters of neurons and numerous fibres were seen in the mesencephalon, in the central gray and the intercollicular nucleus (*Fig. 13A, B*) as well as in the mesencephalic reticular formation. Some neurons were found in the interpeduncular nucleus of the mesencephalon (*Fig. 13D*). This group of cells was composed of medium-sized cells exhibiting a few processes. Some neurons were also visible in the optic tectum. A large number of fibres was observed in the rhombencephalon and in the dorsal pontine tegmentum (at the level of the trochlear nucleus). Numerous SRIF-IR cells occupied both the central gray and the adjacent reticular formation ventrolaterally. These areas seemed to corre-

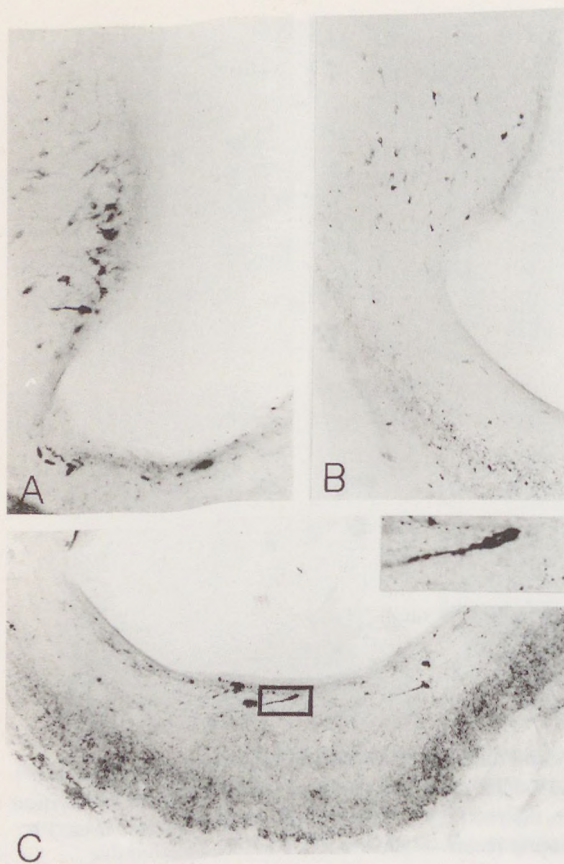


Fig. 11. Coronal sections at the level of the hypothalamic infundibular nucleus. *A*) Periventricular location of SRIF-IR neurons adjacent to the ventral edge of the third ventricle. $\times 260$; *B*) SRIF-IR neurons invade the medial part of the infundibular nucleus. $\times 180$; *C*) SRIF-IR fibres and terminals in the external layer of the anterior median eminence. $\times 180$. The enframed area shows an immunostained cell in the subependymal layer. $\times 400$

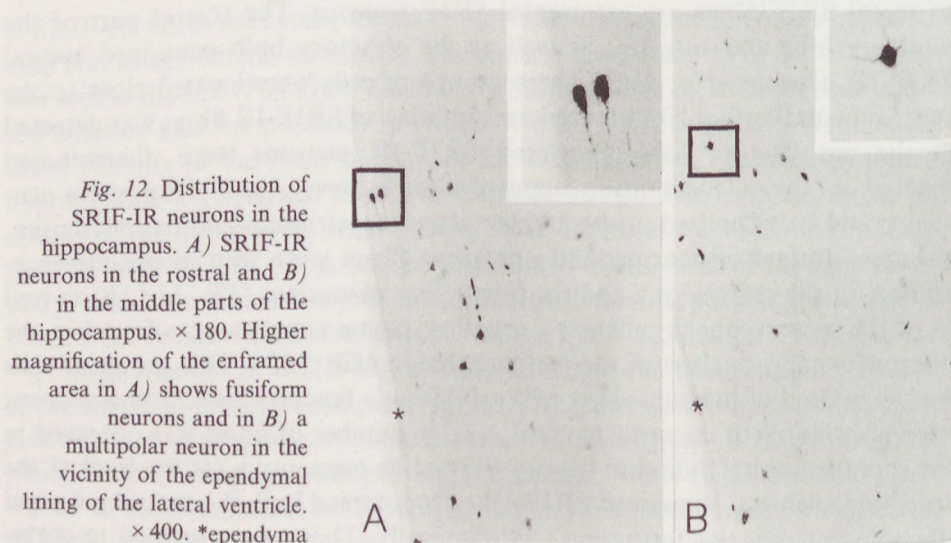


Fig. 12. Distribution of SRIF-IR neurons in the hippocampus. *A*) SRIF-IR neurons in the rostral and *B*) in the middle parts of the hippocampus. $\times 180$. Higher magnification of the enframed area in *A*) shows fusiform neurons and in *B*) a multipolar neuron in the vicinity of the ependymal lining of the lateral ventricle. $\times 400$. *ependyma

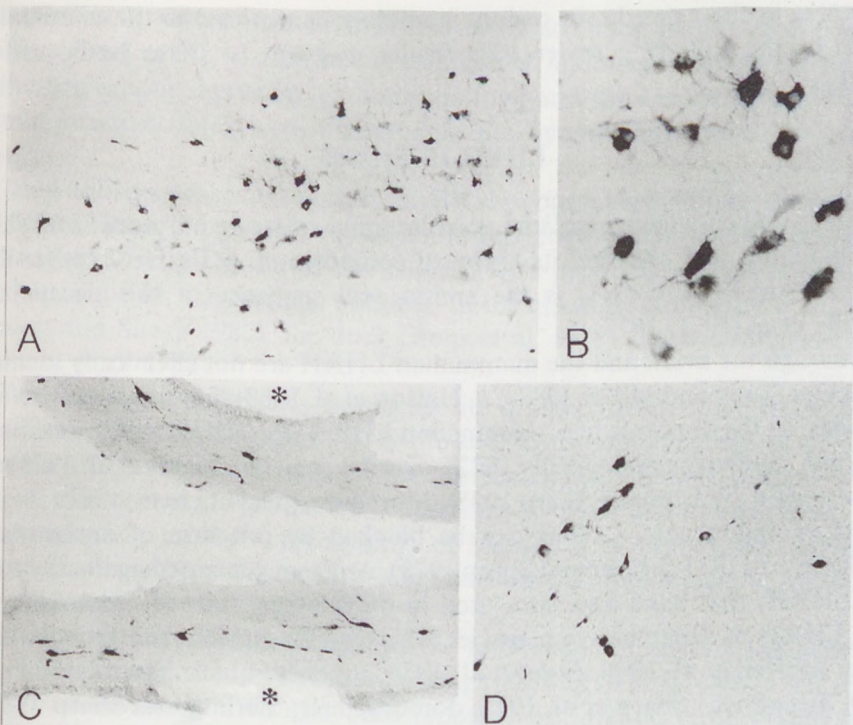


Fig. 13. Distribution of SRIF-IR neurons in extrahypothalamic brain areas. *A)* Numerous SRIF-IR neurons spread in medio-lateral direction in the rostral part of the mesencephalon, in the intercollicular nucleus. $\times 110$; *B)* Higher magnification from the same area. $\times 280$. *C)* SRIF-IR neurons alongside the medial surface of the olfactory bulbs. $\times 110$. *rostral cavities of the ventricles. $\times 110$. *D)* SRIF-IR neurons in the interpenduncular nucleus. $\times 110$

spond to the rostral part of the locus coeruleus defined by Karten and Hodos (1967). Several cells were also found in the ventrolateral part of the parvocellular reticular nucleus. In the medulla oblongata, several SRIF-IR neurons were seen in the nucleus of the solitary tract, some of them spread out laterally into the medullary reticular formation. The raphe nucleus contained a network of SRIF-IR fibres, mostly in its ventral part. Besides these, several areas of the reticular formation were rich in SRIF-IR fibres.

Comparative Aspects

The main goals of our work were 1) to demonstrate the presence of peptidergic neuronal systems in the central nervous system of the chicken and the mallard comparing our results with data of other laboratories obtained on different avian species and 2) to compare the distributional pattern of RH-IR

structures in the avian brain regions homologous to those in the mammalian brain. In the following review the results, relevant to these goals, will be discussed.

LHRH-IR System

Schally and co-workers isolated gonadotropin-releasing hormone from sheep hypothalamus and reported its chemical composition. pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂ is the amino acid sequence of this decapeptide (Schally et al. 1971, 1973).

Although the avian and the mammalian LHRH are not chemically identical molecules (King and Millar 1982*a, b*, Hattori et al. 1980) they are closely related peptides. In the domestic hen, mammalian LHRH (mLHRH) stimulates ovulation (van Tienhoven and Schally 1972), ovarian growth (Reeves et al. 1973) and LH secretion (Wilson and Sharp 1975). Moreover, in laying hens, the biological effects of endogenous LHRH can be blocked by injection of antiserum to synthetic mLHRH (Fraser and Sharp 1978). Antisera generated against synthetic mLHRH, that have also been used in our studies, showed cross-reactivity with LHRH of different avian species (chicken: De Reviere and Dubois 1974, duck: McNeil et al. 1976, Bons et al. 1978, Japanese quail: Davies and Follet 1980, greenfinch: Sharp et al. 1975, domestic hen: Sterling and Sharp 1982).

The avian LHRH was isolated from chicken hypothalamus by King and Millar (1982*a, b*) and Miyamoto et al. (1982*a, b*). The chicken LHRH (cLHRH-I), is structurally different from the mammalian hypothalamic LHRH, arginin in position 8 being replaced by a neutral amino acid, glutamine (Gln₈-LHRH). King and Millar (1982*a, b*) synthesized Gln₈-LHRH and established that it is identical in immunological and biochemical properties with the natural chicken peptide. This peptide induces release of a large amount of LH and a smaller amount of FSH from the pituitary of the rat (Miyamoto et al. 1982*a, b*) and the Japanese quail (Ishii et al. 1984, Hattori et al. 1986). Miyamoto et al. (1984) isolated and characterized a second peptide, the chicken gonadotropin hormone-releasing hormone-II (cGnRH-II), from chicken hypothalamic extracts. cGnRH-II is different from the mLHRH and cLHRH-I in its amino acid sequence, position 5, 7, and 8 being replaced by histidine (His₅), tryptophan (Trp₇) and tyrosine (Tyr₈), respectively. Studies on the ability of the two decapeptides to release luteinizing hormone (LH) in the chicken were also published (Chou et al. 1985, Millar et al. 1986, Sharp et al. 1987).

Immunohistochemical studies using antisera generated against cLHRH-I (Glu₈-LHRH), cGnRH-II (His₅-Trp₇-Tyr₈-GHRH) and mLHRH (Arg₈-LHRH) were carried out in the domestic fowl and the Japanese quail (Mikami et al. 1988). Due to cross-reactivity, perikarya and fibres immunoreactive to anti-cLHRH-I serum were labelled also with mLHRH in both species. The

anti-cGnRH-II serum reacted with neurons distributed in the tuberal hypothalamus and in the rostro-medial area of the midbrain close to the nucleus of the oculomotor nerve in the domestic fowl and the Japanese quail but no immunoreactive fibres were observed in the hypothalamus and the median eminence.

Using antisera generated against mLHRH in our investigations, the majority of LHRH-IR perikarya were found in the preoptico-septal region. This region is thought to be the centre of the avian LHRH production. This notion is in good accord with the findings obtained in the chicken (Hoffmann et al. 1978, Sterling and Sharp 1982), the duck (Bons et al. 1978) and the Japanese quail (Mikami et al. 1988).

There are slight differences among species in the location of LHRH-producing cells in the preoptic region. Only the anterior preoptic nucleus of the quail (Oksche 1978), the dorsal part of the periventricular preoptic nucleus of the mallard (Bons et al. 1978) has been described to show immunoreactivity. Our data contradict, however, the findings of McNeil et al. (1976): no LHRH-IR cell bodies have been observed in the ventromedial hypothalamic nucleus in our studies.

Among extrahypothalamic regions the limbic system, mainly the olfactory bulb and lobe, appeared to be the area being rich in LHRH-IR cells in our study. Similar observations have also been reported by Mikami et al. (1988) using cLHRH-I antibody. A dense network of LHRH-IR fibres and terminals in the OVLT, observed in our experiments, was also demonstrated by Sterling and Sharp (1982) in the domestic fowl and by Oksche (1978) in the quail. It is noteworthy that the periaqueductal gray of the mesencephalon and the nucleus of the oculomotor nerve were found to contain numerous immunopositive fibres in our study. These observations seem to be similar to those concerning cGnRH-II antibodies but perikarya, being shown with cGnRH-II antiserum, failed to show immunoreactivity when stained with cLHRH-I or mLHRH antisera.

Comparing the pattern of distribution of the LHRH neuron system in birds and mammals, a number of parallels could be recognized. The predominant preoptico-septal localization of the LHRH-IR perikarya in birds was confirmed also in the rat by Sétáló et al. (1976), Flerkó et al. (1980) and Merchenthaler et al. (1984). Barry and Carette (1975) obtained similar results in primates. Even the course of the main LHRH fibres tracts in the rat, the preoptico-infundibular tract (Merchenthaler et al. 1980) and preoptico-terminal tract (Kozłowski and Hostetter 1978, Merchenthaler et al. 1980) was similar in birds. Furthermore, LHRH-IR neurons were found in the olfactory bulb of the chicken and a longitudinal fibre system was also described in the olfactory lobe of the hamster (Jennes and Stumpf 1980) and the rat (Réthelyi et al. 1981).

Figure 14 demonstrates the distributional pattern of the LHRH-IR neuronal structures in the avian and mammalian brain. The localization of these struc-

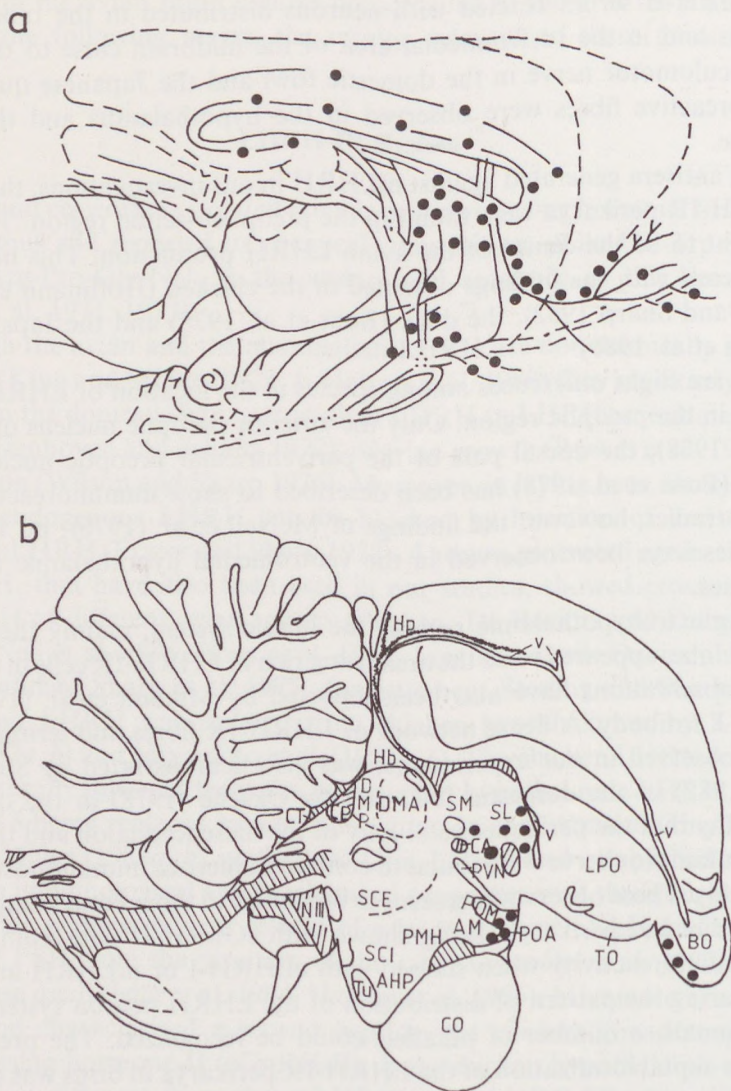


Fig. 14. Diagrams representing the distribution of LHRH-IR neurons in mid-sagittal sections of the rat (a) and the chicken (b) brain (dots). In diagram a, smaller dots represent LHRH-IR terminals, larger dots represent LHRH-IR perikarya according to Merchenthaler et al. (1984). For abbreviations see list on pp. 11-12

tures in the avian brain (b) represents our own findings in the chicken, while the schematic illustration of the rat brain (a) was obtained from the work of Merchenthaler et al. (1984). Both experimental series were performed in our laboratory using the same anti-mLHRH serum.

CRF-IR System

Immunohistochemical data indicate the presence of CRF in the central nervous system of several avian species:

Species	Authors
galliform	
domestic fowl (<i>Gallus domesticus</i>)	Józsa et al. (1984)
Japanese quail (<i>Coturnix coturnix</i>)	Yamada and Mikami (1985) Panzica et al. (1986)
columbiform	
pigeon (<i>Columba livia</i>)	Péczy and Antoni (1984)
passerine	
house sparrow (<i>Passer domesticus</i>) European blackbird (<i>Turdus merula</i>)	Bugnon et al. (1984)
European starling (<i>Sturnus vulgaris</i>) song sparrow (<i>Melospiza melodia</i>)	Ball et al. (1989)

It should be noted that in these studies antisera generated against synthetic ovine CRF were used; a direct comparison between the different avian species could thus be made.

The periventricular-medial hypothalamic location was the main site of CRF-IR perikarya in all investigated avian species. The area, containing the majority of the CRF-IR neural elements, extended rostro-caudally along the wall of the third ventricle up to the infundibular recess, including the medial preoptic nucleus and the paraventricular nucleus. In this respect, only slight differences could be found in numbers, e.g. Panzica et al. (1986) mentioned the infundibular nucleus being one of the main sites of CRF-IR cells (quail), while in our studies only scattered perikarya were detected in this nucleus (chicken). Similar agreement could be drawn regarding the extrahypothalamic localization of the CRF-IR cell bodies. The thalamic nuclei, the accumbens nucleus and the central gray of the mesencephalon were noted as containing the greatest number of CRF-IR cells in all cases listed above. It should be emphasized that no CRF-IR neurons were observed in any avian species in areas homologous with the cerebral cortex of mammals.

Comparable schemes of the localization of CRF-IR neural elements in birds and mammals are shown in *Fig. 15*. These are based upon data on the chicken (*b*; our results) and the rat (*a*; data of Merchenthaler et al. 1982).

Concerning the hypothalamic localization of the CRF-IR neurons, there is a considerable identity between birds and mammals. The parvicellular division of the paraventricular nucleus and the periventricular preoptic nucleus are the most prominent areas containing huge number of CRF-IR neurons also in mammals (rat: Merchenthaler et al. 1982, Bugnon et al. 1982*b*, Bloom et al.

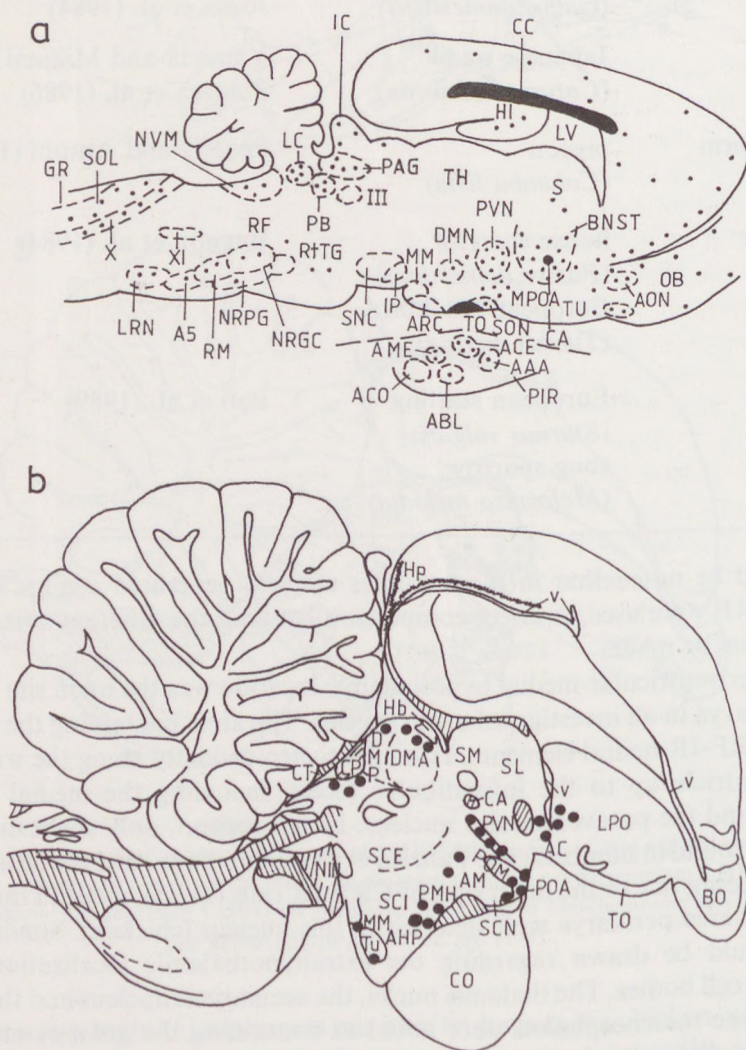


Fig. 15. Schematic drawing showing the distribution of CRF-IR perikarya (dots): *a*) in the brain of colchicine-treated rat [based on data of Merchenthaler et al. (1984)], and *b*) in the chicken brain. For abbreviations see list on pp. 11–12

1982, sheep: Paull et al. 1981). A similarity was observed between birds and mammals regarding the presence of CRF-IR perikarya in the premammillary region and the periaqueductal gray of the mesencephalon as well (Merchenthaler et al. 1982); however, this latter finding of Merchenthaler et al. (1982) is in contrast to the data of Bugnon et al. (1982b), Bloom et al. (1982) and Paull et al. (1981).

The most striking differences in the distribution of CRF-IR neurons were found in the telencephalon and in the rhombencephalon. The CRF system was more widely distributed in the central nervous system of mammals than in birds. In the telencephalon of the chicken, based on our findings, the accumbens nucleus and the parolfactory lobe contained CRF-IR cells only, while, using the same CRF-antiserum, Merchenthaler et al. (1982) could detect CRF-IR cells in the amygdala, the medial septal nucleus, the pyramidal layer of the hippocampus and in the neocortex of the rat. The failure to find any indication of CRF-like immunoreactivity in the areas of the avian brain homologous to the mammalian neocortex, such as the ventral and dorsal hyperstriatum, the neostriatum and the ectostriatum, appeared to be one of the major differences in the distribution of CRF between avian and mammalian taxa. In the brainstem of the rat, the locus coeruleus and nuclei of some cranial nerves (medial vestibular nucleus, nucleus prepositus of the hypoglossal nerve) also revealed CRF-IR cell bodies in the rat, while no CRF-IR structures were reported in birds in homologous regions.

CRF-IR fibres were extensively distributed in several mammalian and avian species; it appeared evident, however, that the CRF-IR fibre system was more ubiquitous in the rat than in the chicken.

TRH-IR System

Interavian comparison, concerning the distribution of the TRH-IR neuron system, has been hampered because of the lack of sufficient immunocytochemical data. So far, the report of Thommes et al. (1985) was found to be the only one dealing with the TRH system in birds (chicken embryo). The embryonic and the adult central nervous system cannot be considered identical structures in many respects, therefore this study will be discussed in relation to the ontogenetic development of the RH systems in Chapter 4 of this monograph.

In mammals, most of the studies on the localization of the TRH-IR system have been performed on rats. Therefore, in the present overview the rat represents the mammalian model. *Figure 16* summarizes the similarities and differences of the TRH-IR system in the rat (*a*; Merchenthaler et al. 1988) and the mallard (*b*; our studies).

At first sight, it appears conspicuous that the TRH-IR neuron system is as

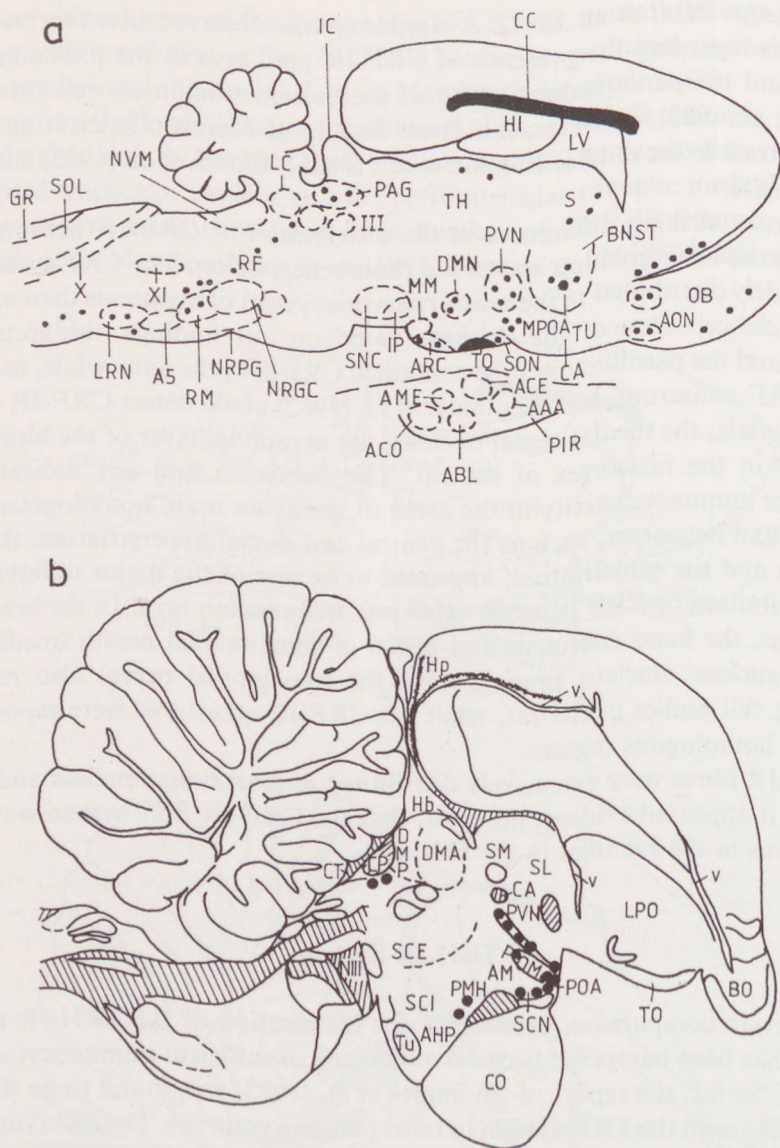


Fig. 16. Schematic representation of TRH-IR neurons *a*) in a paramedian-sagittal section of the rat brain [based on data of Merchenthaler et al. (1988) and *b*) in a sagittal section of the avian brain. For abbreviations see list on pp. 11–12

widely distributed in birds as in mammals. A full concordance is evident concerning the main hypothalamic sites of localization of TRH-IR perikarya. The parvocellular part of the paraventricular nucleus and the ventral preoptic area are the main sites containing the majority of these neurons both in birds and mammals. This was observed in the rat by several authors (Choy and

Watkins 1977, Johansson and Hökfelt 1980, Leachan and Jackson 1982, Merchenthaler et al. 1988). Smaller TRH-cell groups were detected in the suprachiasmatic, lateral and posterior hypothalamic nuclei in both animal models. It appears, however, that the hypothalamic distribution of the TRH-IR perikarya is more diffuse in the rat than in the mallard. It is worth mentioning that no immunoreactivity was observed in the magnocellular division of the paraventricular nucleus in birds, whereas TRH-IR perikarya were occasionally seen in this region in mammals. Besides this difference, the magnocellular supraoptic-, the dorsomedial hypothalamic and the arcuate nuclei of the rat possessed TRH-IR neurons (Merchenthaler et al. 1988) in contrast to the lack of immunoreactivity of these nuclei in the mallard.

Among extrahypothalamic sites, only the diagonal band of Broca and the central gray of the mesencephalon are the two regions where TRH-IR perikarya were found both in birds and mammals. It should be mentioned that clearly defined groups of TRH-IR neurons were rarely observed in the extrahypothalamic brain regions of the mallard. In contrast, many areas of the limbic system, such as the anterior olfactory nucleus, the olfactory bulb, the medial septum, the septohypothalamic nucleus, the bed nucleus of the stria terminalis, the accumbens nucleus, contained TRH-IR cells in the rat (Merchenthaler et al. 1988). Furthermore, TRH-IR cells could be detected nearly throughout the entire brainstem of the mammals. Smaller or larger TRH-IR cell clusters were present also in the dorsal- and median raphe nuclei, the nuclei raphe magnus and pallidus, the reticular formation of the mesencephalon, the paragigantocellular nucleus of the pons and the lateral reticular nucleus of the medulla oblongata (Merchenthaler et al. 1988), while the corresponding areas in birds did not contain detectable amounts of immunoreactive material. It should be noted that, in contrast to the CRF system in mammals, neither the cerebral cortex of the rat nor the homologous regions of the duck contained TRH-IR cells.

Since the possible neuronal projections of the different hypothalamic and extrahypothalamic RH- and IH-IR neurons are discussed in Chapter 5 (p. 71) of this book, here we omit the comparative analysis of the distribution of TRH-IR fibres and terminals.

SRIF-IR System

It is well established that the circulating growth hormone (GH) level of birds is age related, being high in young birds and low in adults. A fall in the concentration of plasma GH was demonstrated in growing ducks (Foltzer et al. 1981), chickens (Harvey et al. 1979a, Scanes and Harvey 1981), turkey (Harvey et al. 1979b), geese (Scanes et al. 1979) and ring doves (Scanes and Balthazart

1981). Plasma GH responses to hypothalamic GH-releasing factors in anaesthetized adults are less than those in young birds and are almost absent in conscious adults (Scanes and Harvey 1984). The half-life of GH in peripheral plasma is increased rather than decreased in adult birds (Hall et al. 1985), therefore the maturational decline in the circulating GH level is not due to a change in the rate of GH metabolism. The development of an inhibitory control mechanism for GH secretion during growth may contribute to the decrease in plasma GH levels. The findings, that the adult pituitary gland is not deficient in somatotrophs (Malamed et al. 1985) and is responsive to provocative stimuli *in vitro* (Hall et al. 1985), support this hypothesis. The inhibitory mechanism responsible for the decline in GH secretion during growth can peripherally be influenced through inhibition by thyroid, pancreatic, adrenal or gonadal hormones (Harvey 1983). Castration, however, does not affect the age-related decline in GH secretion (Scanes and Johnson 1984). Plasma concentrations of thyroxine, triiodothyronine, insulin, glucagon and corticosterone are not increased during growth (Harvey and Phillips 1980, Foltzer et al. 1981, Sterling et al. 1984). Hypothalamic and/or peripheral SRIF may, however, participate in the inhibition of GH during growth.

SRIF, initially identified in the ovine hypothalamus as a GH release inhibiting hormone (Brezeau et al. 1973) is present in the peripheral plasma and in the brain of ducks (Calas et al. 1975, Blähser 1984, Di Scala-Guenot et al. 1984). Both somatostatin-14 and -28 are potent inhibitors of basal GH secretion in ducklings (Strosser et al. 1984).

Topography of SRIF-IR neurons in the chicken and rat brain is demonstrated in schematic drawings (*Fig. 17*) as judged from the literature and our own data. Mammalian model *a* was taken from the review of Nieuwenhuys (1985), *b* represents the avian model based on our data.

Interavian comparison comprises the domestic fowl (Dubois 1976 and our data), the mallard (Blähser 1978), the grass parakeet (Shiosaka et al. 1981, Takatsuki et al. 1981) and the pigeon (Anderson and Reiner 1990). In contrast to the RHs, there were conspicuous differences in the intrahypothalamic localization of the SRIF-IR perikarya described in the investigated avian species. In the domestic mallard, the supraoptic and paraventricular hypothalamic nuclei and the preoptic region contain the vast majority of the SRIF-IR neurons (Blähser 1978). Mainly concordant data were reported by Dubois (1976) describing two distinct SRIF-IR systems in the cock: the parvicellular system located around the preoptic recess of the third ventricle and the magnocellular system that comprises the supraoptic and paraventricular hypothalamic nuclei. Our findings are in good accordance with data reporting accumulation of parvocellular SRIF-IR neurons around the preoptic recess of the third ventricle and in the paraventricular nucleus, but no magnocellular elements were stained in our studies. In the grass parakeet, however, the nucleus medialis hypothalami

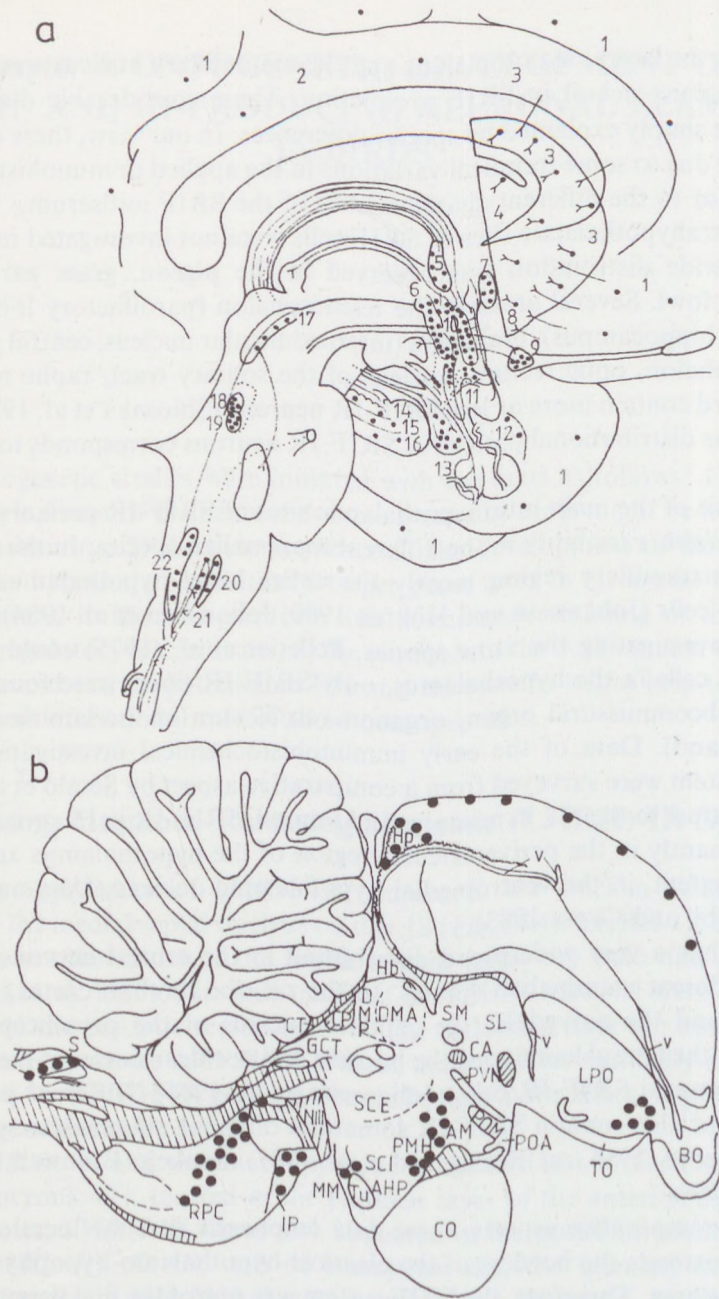


Fig. 17. Diagrams representing the distribution of SRIF-IR neurons: a) in mammalian and b) in avian brains. [Diagram a) was taken from: Nieuwenhuys (1985)]. Numbered areas a): 1: Neocortex; 2: Cingulate gyrus; 3: Caudate nucleus; 4: Putamen; 5: Nucleus interstitialis striae terminalis; 6: Paraventricular nucleus; 7: Nucleus septi lateralis; 8: Nucleus accumbens; 9: Nucleus olfactorius anterior + olfactory tubercle; 10: Preoptico-hypothalamic SRIF-IR group; 11: Suprachiasmatic nucleus; 12: Prepiriform cortex; 13: Amygdaloid body; 14: Dentate fascia; 15: Cornu Ammonis; 16: Subiculum; 17: Central gray of the mesencephalon; 18: Nucleus lemnisci lateralis dorsalis; 19: Nucleus parabrachialis medialis; 20: Ambiguous nucleus; 21: Substantia gelatinosa nuclei spinalis nervi trigemini pars caudalis; 22: Solitary nucleus. For abbreviations see list on pp. 11-12

posterior, the lateral hypothalamus and the mammillary nucleus were found to be the regions richest in SRIF production. These considerable discrepancies cannot be simply explained by species differences. In our view, these differences are either due to some technical variations in the applied immunohistochemical methods or to the different characteristics of the SRIF antiserum.

The extrahypothalamic sites of SRIF cells were not investigated in the duck, while a wide distribution was observed in the pigeon, grass parakeet and domestic fowl. Several areas in the telencephalon (parolfactory lobe, palaeostriatum, hippocampus), brainstem (interpeduncular nucleus, central gray of the mesencephalon, optic tectum, nucleus of the solitary tract, raphe nuclei) and spinal cord contain more or less SRIF-IR neurons (Shiosaka et al. 1981). In our studies the distributional pattern of SRIF-IR neurons corresponds to the above regions.

Analysis of the main intrahypothalamic sites of SRIF-IR perikarya revealed a considerable variability in the different mammalian species. In the rat, except for the mammillary region, nearly the entire basal hypothalamus, contains SRIF-IR cells (Johansson and Hökfelt 1980, Johansson et al. 1984). A decade earlier, investigating the same species, Pelletier et al. (1975) could not detect SRIF-IR cells in the hypothalamus, only SRIF-IR fibres were found in some areas (subcommissural organ, organon vasculosum of the lamina terminalis, pineal gland). Data of the early immunohistochemical investigations of the SRIF system were surveyed from a comparative aspect by Sétáló et al. (1978*b*).

In contrast to the rat, in primates and human, SRIF-IR perikarya are located predominantly in the periventricular region of the hypothalamus and, only to a lesser extent, in the ventromedial hypothalamic nucleus (Désy and Pelletier 1977, Filby and Gross 1983).

SRIF has a very widespread distribution in the central nervous system of many different mammalian species. In the rat, the cerebral cortex, the hippocampus and the amygdala, the caudate nucleus of the prosencephalon, the region of the lateral lemniscus, the pontine and cochlear nuclei of the brainstem contain several SRIF-IR cells (Johansson et al. 1984). Different parts of the spinal cord also contain SRIF-IR somata in different mammalian species (cat: Krukoff et al. 1986, rat: Dalsgaard et al. 1981, monkey: Burnweit and Forssmann 1979).

From comparative aspects, these data emphasize that the location of SRIF neurons extends the borders of the classical hypothalamo-hypophyseal neuroendocrine area. Therefore, the SRIF system was one of the first assumed to have a bifunctional role, namely the regulation of tropic hormone (GH) synthesis and/or release and a neurotransmitter function (Krisch 1977, Blähser 1978, Johansson et al. 1984). This aspect will be discussed in detail in Chapter 5 (p. 71).

Chapter 4. ONTOGENETIC DEVELOPMENT OF THE RH- AND IH-PRODUCING NEURONAL ELEMENTS IN BIRDS

Our ontogenetic studies were initiated with the aims as follows: 1) all data on the development of the neuroendocrine system of birds might be helpful for poultry husbandry; 2) the time sequence of the development of the different levels in the hypothalamo-pituitary-target gland axes in birds and mammals might be useful in the interpretation and better understanding of the neuroendocrine regulatory mechanisms; 3) the sequence in the development of the hypothalamic and extrahypothalamic RH and IH systems might help to explain the proposed bifunctional role of these neuropeptides.

Luteinizing Hormone-Releasing Hormone (LHRH)-IR System

The first appearance of LHRH-IR neurons in the chicken embryos was detected in the medial septal nucleus on day 18 (*Fig. 18A*). LHRH-IR cells were also visible in other parts of the limbic system (olfactory bulb and olfactory lobe) (Mess et al. 1983). The first traces of LHRH-IR material were observed on day 18 in the preoptic region as well. The following days the number and staining intensity of the LHRH-IR cells of these regions markedly increased. On day 19, a few LHRH-IR perikarya could be seen in the lateral hypothalamus and the suprachiasmatic area.

LHRH-IR fibres and terminals appeared in the ME not earlier than day 18 during embryonic life, located in the palisade layer of the anterior division of the ME. Later, LHRH-IR fibres also appeared in the posterior portion of the ME (*Fig. 18C, D*). At the end of embryonic life, the intensity of LHRH immunoreactivity in this fibre system considerably increased. In the medial septal area and around the OVLT a dense network of LHRH-IR fibres and terminals was detected during the early posthatching period.

There was no significant difference in the appearance of LHRH-IR neuronal elements in the hypothalamic and extrahypothalamic areas. LHRH-IR cells in the preoptic area and the medial septal nucleus, as well as LHRH-IR fibres and

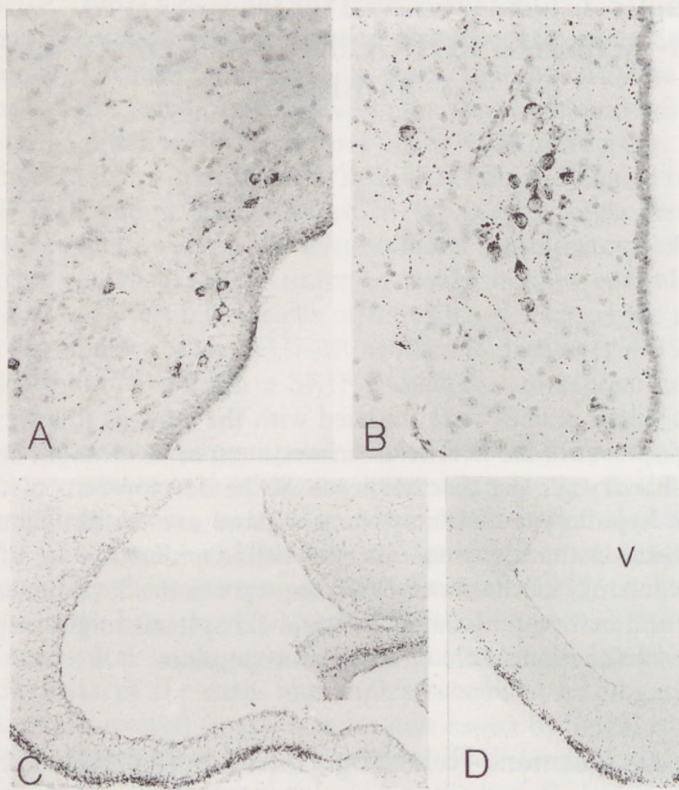


Fig. 18. LHRH-IR neurons in the developing chicken brain. *A)* LHRH-IR cell bodies and fibres in the medial septal nucleus on day 19. Sagittal section. $\times 250$. *B)* LHRH-IR neurons in this area progressively increase in number and size with advancing age as seen in the adult. $\times 110$; *C)* LHRH-IR terminals in the median eminence on day 18. Sagittal section. $\times 200$; *D)* LHRH-IR terminals in the median eminence on day 21. The LHRH-IR axons and terminals are seen in the palisade layer of the external zone. Coronal section. V: third ventricle. $\times 200$

terminals in the ME were detectable nearly simultaneously on day 18. Although, very few and weakly stained LHRH cells could be detected in the preoptic area one day earlier (day 17). This slight difference cannot be considered to have functional significance.

The relationship between the onset of hypothalamic LHRH and pituitary LH production will be discussed on p 75.

In our studies, the onset of LHRH production in the brain during development was the last one among the investigated RHs and IHs. Extracerebral structures were outside the scope of this review. Therefore, the olfactory placode which, is thought to be the site containing the precursor cells of LHRH neurons (Akutsu et al. 1992), was not examined. A significantly earlier appearance of LHRH was found in this area, this crucial question will be discussed on p 61.

Corticotropin-Releasing Factor (CRF)-IR System

In our study, CRF-IR perikarya were not seen in the brain of the chick embryo before day 14 of incubation. On day 14, scattered CRF-IR cells could be detected in the midline of the hypothalamus belonging to the periventricular region of the paraventricular nucleus (*Fig. 19A*). On the same day, another group of cells, composed of few, weakly immunoreactive elements, was also

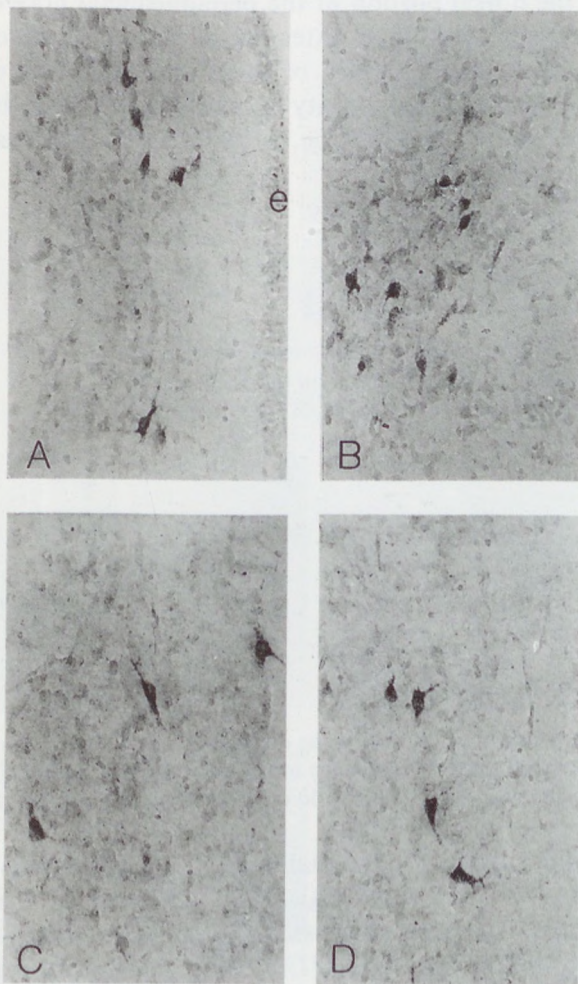


Fig. 19. CRF-IR perikarya in the hypothalamus of the developing chicken embryo. CRF-containing, weakly stained small perikarya are located. *A*) in the periventricular portion of the paraventricular nucleus on day 14; *e*: ependyma. *B*) in the region of the nucleus medialis hypothalami posterior on the same day; *C*) in the medial portion of the paraventricular nucleus on day 15 and *D*) on day 16 of incubation. $\times 160$

observed in the posterior hypothalamic nucleus (*Fig. 19B*). The number of cells and the intensity of immunoreactivity rapidly increased in both groups of cells on days 15 and 16 (*Fig. 19C, D*).

The majority of the CRF-IR perikarya was restricted to the above-mentioned two nuclei of the hypothalamus in the embryonic chicken brain. However, some extrahypothalamic regions also revealed CRF-IR cells during embryonic life. The first extrahypothalamic IR-perikarya appeared around the wall of the cerebral aqueduct on the 15th day of incubation (*Fig. 20A*). These large cells were located in the dorsal portion of the periaqueductal gray on the anterior part of the mesencephalon. They extended in ventral direction towards the caudal part of the midbrain. A few of these cells were located immediately beneath the tanycytes and in the vicinity of capillaries. The number of these cells steadily increased from day 15 on (*Fig. 20B*). A further increase was observed also after hatching (*Fig. 20C*).

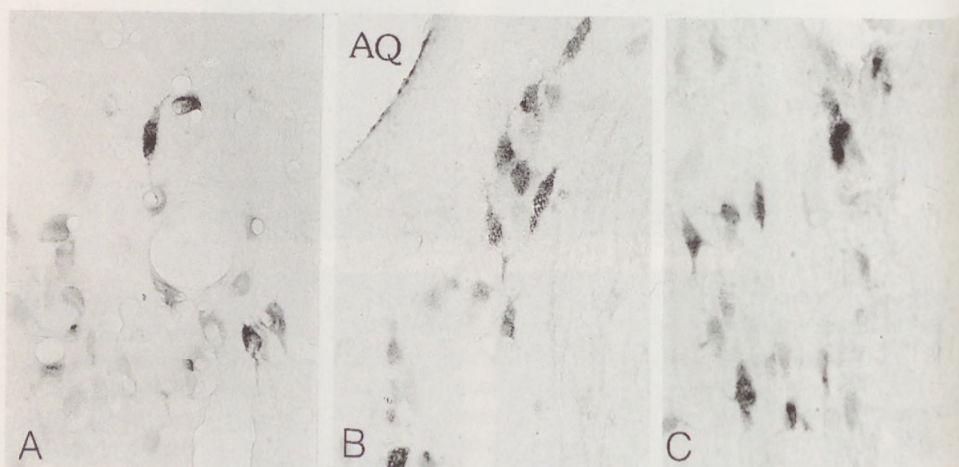


Fig. 20. CRF-IR perikarya demonstrable in the extrahypothalamic brain regions of the chicken brain during development. Large cells are seen in the mesencephalic central gray *A*) on day 15; *B*) on day 2 after hatching and *C*) on day 30 after hatching. AQ: cerebral aqueduct. $\times 300$

CRF-IR neurons appeared in the thalamus, in the accumbens nucleus and in the septal region only after hatching. In the dorsomedial thalamic nucleus, around the medullary stria, medium-sized perikarya were immunostained on the second day after hatching (*Fig. 21A*). The number and staining intensity increased on the following posthatching days (*Fig. 21B*). After hatching, on day 7, scattered CRF-IR perikarya were detected in the nucleus accumbens (*Fig. 21C*). No considerable increase in the number of these small-sized cells was detected in the forthcoming days (*Fig. 21D*). Caudally, these cells extended gradually towards the septal region.

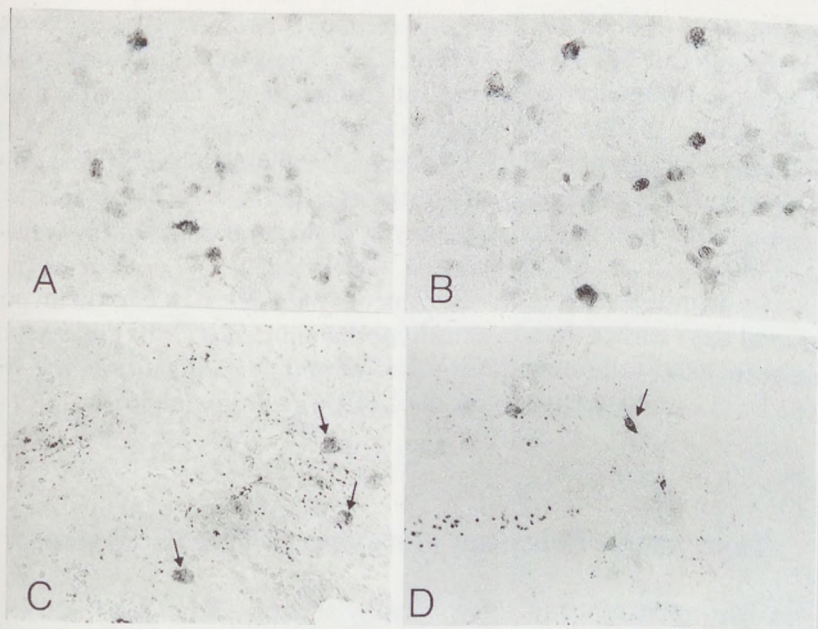


Fig. 21. CRF-IR perikarya and fibres in the nucleus dorsomedialis thalami on day 2 after hatching (A), on day 30 after hatching (B) and in the nucleus accumbens (arrows) on day 7 after hatching (C) and on day 30 (arrow) after hatching (D). $\times 300$

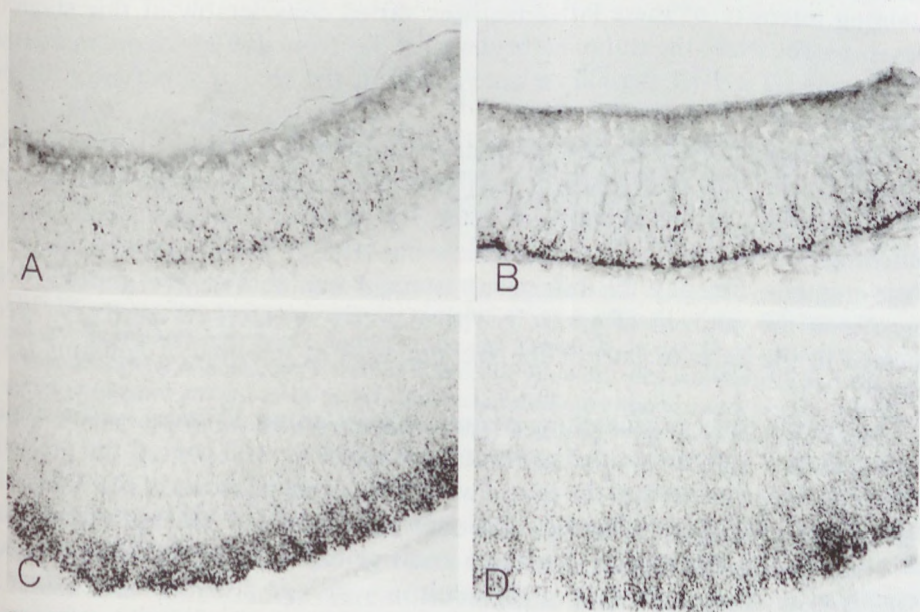


Fig. 22. Frontal sections through the anterior median eminence of chicken embryos. CRF-IR fibres and terminals are seen in four different stages of development. $\times 200$. A) A few CRF-IR neural elements are seen in the external zone of the median eminence on day 15; B) Their number increases slightly on day 16; C) Densely packed immunoreactive elements, indicating a filled system, are accumulated in the external zone of the median eminence on day 2 after hatching; D) A slight decrease in the number of fibres can be observed in the external zone on day 18 after hatching

The first signs of CRF-IR fibres and terminals were detected in the external zone of the anterior ME on day 14 (*Fig. 22A*). These weakly stained axons were found in close proximity to the ventral surface of the ME. On the following days, the number of CRF-IR fibres was slightly increased (*Fig. 22B*). A further slow increase in the number of CRF fibres and terminals was observed only after hatching (*Fig. 22C*), predominantly in the external zone of the ME. Curiously enough, up to the 18th posthatching day, a slight decrease of immunoreactivity was seen in both zones of the ME (*Fig. 22D*).

As it was demonstrated, CRF-IR elements were detectable in the hypothalamus several days earlier than in extrahypothalamic sites (with the exception of the mesencephalic cell-population). This developmental pattern was slightly different from our observations on the LHRH-neuron systems.

Thyrotropin-Releasing Hormone (TRH)-IR System

The earliest appearance of a few, weakly stained TRH-IR perikarya in the mallard embryo was detected in the periventricular region of the middle hypothalamus as early as day 14 of incubation. On day 20, a small group of TRH-IR cells was noted in the developing paraventricular nucleus (*Fig. 23A*). The staining intensity of these latter neurons varied considerably at this stage of development. Near the end of embryonic life, i.e. from day 25–26 on, numerous well-stained TRH-IR perikarya were found in the medial subdivision of the paraventricular nucleus (*Fig. 23B*).

In the preoptic area a similar pattern could be observed in the appearance of TRH-IR cells. The first TRH-IR perikarya were detected in this area on day 16 of incubation. The number of these IR neurons increased steadily until hatching. Up to day 20, there was a slow but later on a more rapid increase in their number. On day 22, intensively stained immunoreactive material was present in the neurons (*Fig. 23C*). The majority of TRH-IR perikarya were located in the anterior part of the preoptic area at the time of hatching (*Fig. 23D*).

During the early posthatching period, a large group of small fusiform and round-shaped immunostained cells was seen in the ventral part of the preoptic area (*Fig. 24B*) and around the dorsal wall of the preoptic recess of the 7-day-old duck brain (*Fig. 24A*). The distributional pattern of the TRH-IR perikarya, observed in the above-mentioned two areas at the early posthatching period, resembled in all respects that of the adult.

On day 21, a few, large, elongated TRH-perikarya appeared in the supraoptic nucleus of the hypothalamus (*Fig. 23E, F, G*). It should be mentioned, however, that these cells could be detected only up to hatching. In the posthatching period

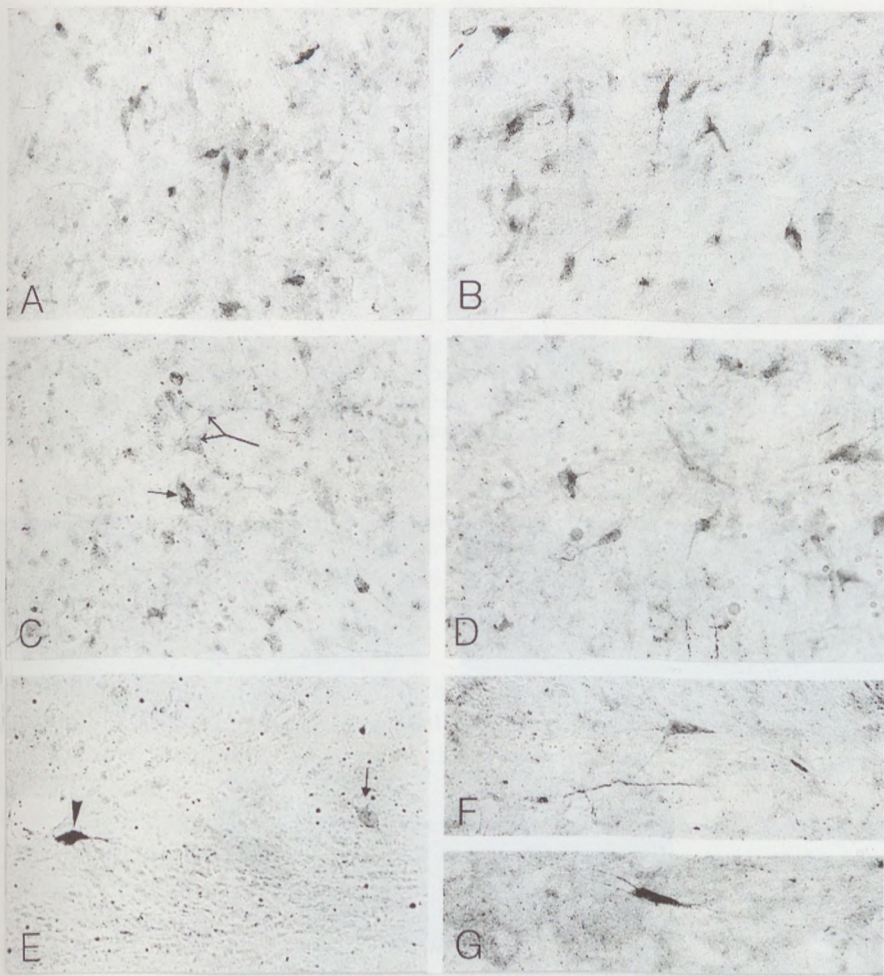


Fig. 23. TRH-IR perikarya in different regions of the hypothalamus of mallard embryos. $\times 250$.

TRH-IR perikarya in the paraventricular nucleus. *A*) on day 20; *B*) on day 26; TRH-IR perikarya in the anterior preoptic region, *C*) on day 22 and *D*) on day 27. Note the strong immunoreactivity of a neuron (arrow), in contrast to the weak immunoreactivity in other neurons (bifurcated arrow); *E*) A densely immunolabelled (arrowhead) and a weakly stained perikaryon (arrow) in the supraoptic region on day 23; *F*), *G*) Large perikarya in the same region before hatching

and in adult age, no TRH-IR cells were seen in the supraoptic nucleus. The explanation of this deviant finding needs further experimental elucidation.

Immunoreactive perikarya were not detectable in the brainstem during embryonic development. TRH-IR neurons in the central gray of the mesencephalon, described in the adult (Józsa et al. 1988), were visible only at the end of embryonic life.

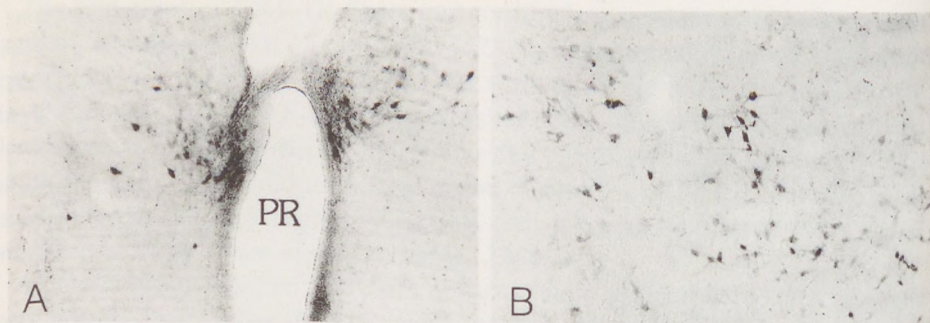


Fig. 24. Coronal sections through the preoptic region of a 7-day-old mallard after colchicine treatment. $\times 110$. *A*) Numerous TRH-IR cell-bodies are present around the wall of the preoptic recess (PR); *B*) A great number of TRH-IR perikarya spread in medio-lateral direction in the anterior preoptic region

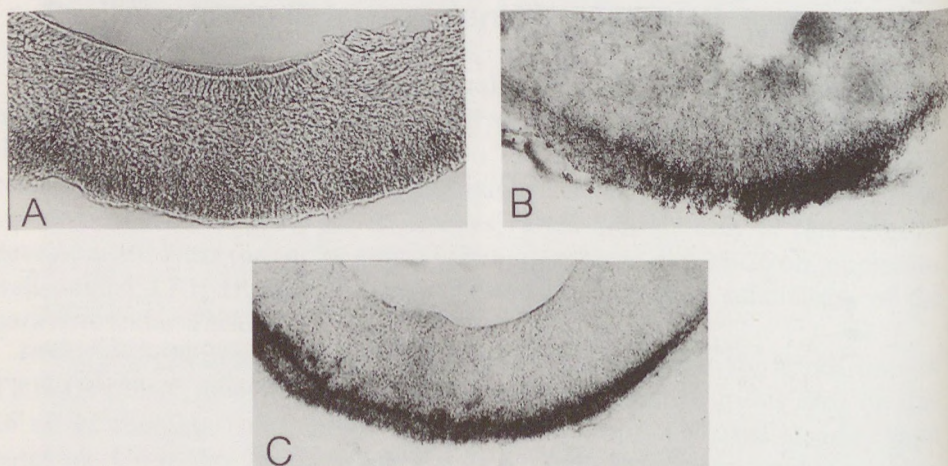


Fig. 25. Coronal sections through the anterior median eminence of mallard embryos and a 7-day-old mallard. $\times 200$. *A*) Few, dot-like TRH-IR structures (arrows) are seen on day 20; *B*) TRH-IR fibres and terminals are concentrated mainly in the middle portion of the external zone on day 27; *C*) TRH-IR fibres are evenly distributed in the external zone of the median eminence on day 7 after hatching

TRH-IR fibres and terminals in the ME were first visualized on day 20 of development. Only a few, dot-like structures could be seen in the external zone of the anterior ME (*Fig. 25A*). The number of TRH-IR fibres markedly increased in the subsequent days. Just before hatching, on day 27 of incubation (*Fig. 25B*) and in the early posthatching period (7 days after hatching) (*Fig. 25C*) numerous TRH-IR fibres and terminals accumulated in the anterior part of the ME. In this perinatal period, a few TRH fibres appeared also in the posterior part of the ME.

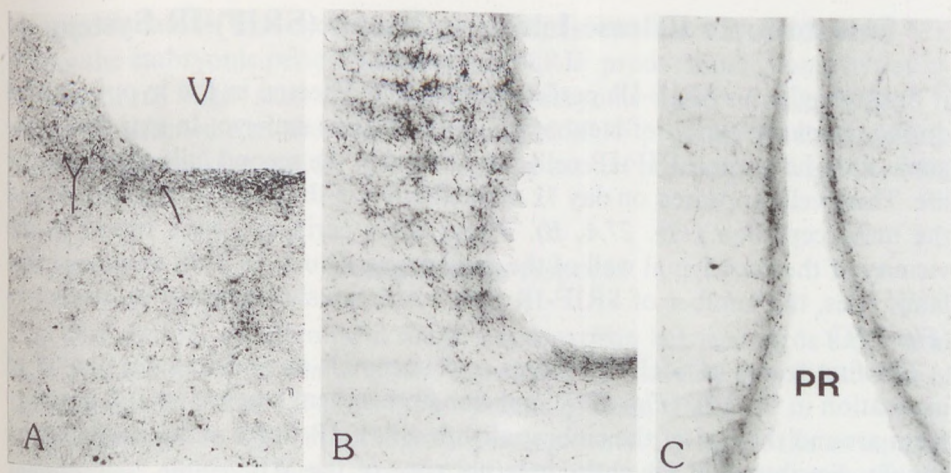


Fig. 26. Coronal sections showing the distribution of TRH-IR fibres in different brain areas of mallard embryos. $\times 200$. *A*) TRH-IR fibres in the lateral septum on day 20. Note IR fibres (arrows) in the subependymal layer around the ventral border of the lateral ventricle (V); *B*) TRH-IR fibres in the lateral septum on day 26; *C*) Numerous TRH-IR fibres in the preoptic region in close vicinity to the wall of the preoptic recess (PR) on day 26

During the second half of embryonic life, fine TRH fibres were present throughout the hypothalamus, especially in the vicinity of TRH-IR perikarya. Some fibres were running adjacent to the ependymal wall of the third ventricle (Fig. 26C).

In extrahypothalamic regions, scattered TRH-IR fibres were observed from day 20 on. A few fine fibres were seen in the subependymal zone of the lateral septal area (Fig. 26A). The number of these fibres continuously increased up to the time of hatching (Fig. 26B). After hatching, there was a further dramatic increase in this region resulting in a dense network of fibres on the 7th post-hatching day.

Other extrahypothalamic brain areas, like the parolfactory lobe, the nucleus accumbens, the hippocampus, the dorsal thalamic nucleus or the mesencephalic central gray, which are relatively rich in TRH fibres in adult age, contained only a few scattered IR fibres during the second half of embryonic life. These fibres became also more numerous after hatching, but their density did not reach the gradient observed in the lateral septum.

TRH-IR neurons were visible in the hypothalamic nuclei earlier than in the extrahypothalamic brain regions. A similar time sequence was observed also in the case of CRF neurons, but this shift was more expressed in the TRH than in the CRF system.

Somatotropin Release-Inhibiting Factor (SRIF)-IR System

Surprisingly, no SRIF-IR perikarya could be detected in the hypothalamus during the entire period of incubation in the chicken embryo. In extrahypothalamic sites, however, SRIF-IR cells were found in the second half of embryonic life. These cells appeared on day 12 of incubation in the pontine tegmentum and the mesencephalon (Fig. 27A, B). These latter perikarya were found in the vicinity of the ependymal wall of the cerebral aqueduct. In both extrahypothalamic sites, the number of SRIF-IR cells continuously increased up to day 18 (Fig. 27C).

The first traces of SRIF-IR fibres and terminals were found on day 18 of incubation in the ME (Fig. 27D), but this fibre system reached its full development around the end of the embryonic life. SRIF-IR fibres and terminals were equally distributed in the entire external zone of the ME.

In contrast to the RH systems, SRIF-IR neurons appeared first in the extrahypothalamic regions (in the brainstem) and significantly earlier than in

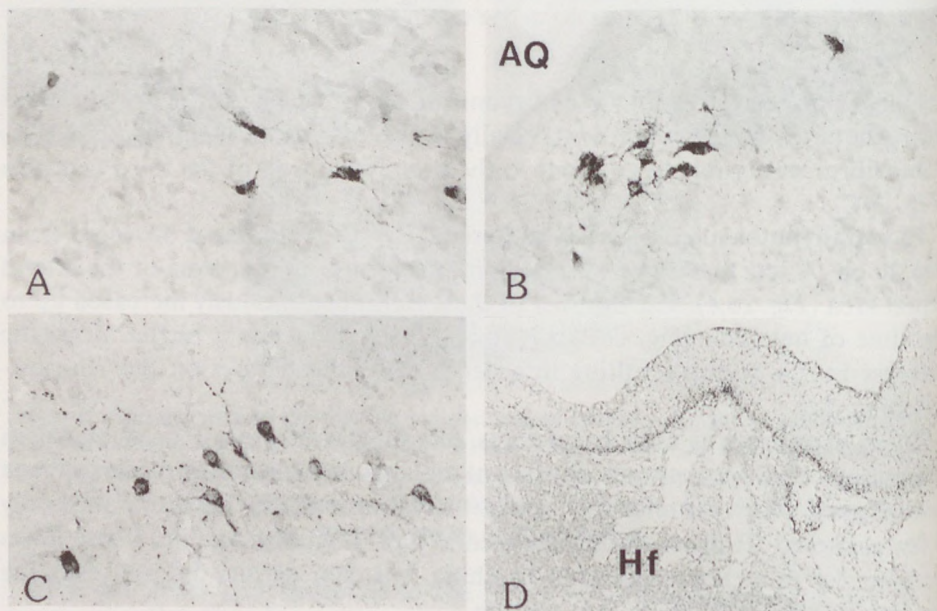


Fig. 27. SRIF-IR neurons in the brain of chicken embryos. A) Multipolar cells are visible in the tegmentum of pons as early as day 12. $\times 200$; B) Another group of cells in the same age is found in the vicinity of the cerebral aqueduct in the central gray of the mesencephalon. AQ: cerebral aqueduct. $\times 200$; C) Immunoreactive cells are seen in the tegmentum of pons on day 18. Note the remarkable increase of SRIF-IR cells. $\times 200$. D) Sagittal section of the median eminence on day 21. Note the small amount of SRIF-IR material in the external zone in close proximity to the superficial primary plexus of the hypothalamo-hypophyseal portal capillaries. Hf: pituitary gland. $\times 200$

the hypothalamus. However, the appearance of some SRIF-IR fibres in the ME during the embryonic period indicated that SRIF production in some hypothalamic neurons might start already at these embryonic stages of development. It might be assumed that the minute amount of SRIF produced in the hypothalamic perikarya were rapidly transported via axonal processes toward the ME.

Comparative Aspects

In this section, we attempt to make a comparison between birds and mammals concerning some characteristic aspects of the developmental patterns of the different RH- and IH-containing systems of their CNS.

The findings on the chronological development of RHs in the avian and mammalian embryos were summarized in *Tables III* and *IV*, respectively. Evidently, the list is not complete, only some representative examples have been collected. The comparison of these findings, most of them obtained by immunocytochemical techniques in several laboratories in the same period (from 1977 to 1988) led to the conclusion that immunoreactive RH neurons appear in the hypothalamus earlier than in extrahypothalamic brain regions or at the same time. This notion, however, has to be reconsidered in the light of some recent data: Schwanzel-Fukuda and Pfaff (1989) and Wray et al. (1989*a, b*) have stated for the first time that neurons expressing LHRH are initially detectable in the olfactory placode of the mouse (on day 11.5) prior to their appearance in the brain. Recently, similar observations have been found also in the chicken embryo by employing a very sensitive method (on day 3; Murakami et al. 1991). At this stage of development, the LHRH cells migrate from the olfactory placode within the axonal fascicles of the olfactory and vomeronasal nerves and penetrate into the forebrain. In the chicken embryo, the migrating cells were detectable both in the septal and preoptic areas already on day 11 (Akutsu et al. 1992). In the mouse, by day 16.5 most cells had completed their migration and they remained distributed both in the hypothalamic (anterior hypothalamic area) and extrahypothalamic brain regions (septo-preoptic area) in the later stages of development (Schwanzel-Fukuda and Pfaff 1989, Wray et al. 1989*a, b*). In the rat, although the first identifiable LHRH cells were present on day 15 in the olfactory bulb (Jennes 1989), no IR axons were detectable in the median eminence before day 19 (Araki et al. 1975, Kawano et al. 1980, Ibata et al. 1981, Jennes 1989). It is not known whether LHRH neurons, projecting to the median eminence, do not express LHRH before they have detectable amount of IR material or the rate of secretion of LHRH is so high that insufficient amounts of the peptide remain in the cell to be detected by immunocytochemistry. LHRH was measured in the amniotic fluid of the rat fetus by radioimmunoassay already from day 12 on, with a maximum concentration

Table III

Day of appearance of RH- and IH-RH perikarya in the hypothalamus and in extrahypothalamic brain areas of the avian embryos

Peptide	Species	Day of hypothalamic appearance	Hypothalamic localization	Day of extrahypothalamic appearance	Extrahypothalamic localization	References
LHRH	chicken	18	preoptic area	18	medial septal nucleus	Mess et al. (1983)
		—		5.5	forebrain	Woods et al. (1985)
		—		3	olfactory placode	Murakami et al. (1991)
	mallard	14	preoptic area	14	septum	Blähser and Heinrichs (1982)
		11	preoptic area	11	septum	Akutsu et al. (1992)
		20	preoptic area caudobasal hypothalamus	25	dorsal septum	Blähser and Heinrichs (1982)
	quail	15	preoptic area	15	septum	Blähser and Heinrichs (1982)
CRF	chicken	14	paraventricular nucleus	15	central gray of the mesencephalon	Józsa et al. (1986)
TRH	chicken			4.5	forebrain	Thommes et al. (1985)
	mallard	14	periventricular middle hypothalamus	28	central gray of the mesencephalon	Józsa et al. (1989)
SRIF	chicken	after hatching	—	12	pontine tegmentum	Mess et al. (1983)
				7	pontine tegmentum	Blähser and Heinrichs 1982
	mallard	after hatching	—	15	paragigantocellular nucleus	Blähser and Heinrichs (1982)
	quail	after hatching	—	11	paragigantocellular nucleus	Blähser and Heinrichs (1982)

on day 16, although the source of the measured peptide is not clear yet (Jennes 1990). The majority of LHRH cells in the nasal septum do not process the LHRH precursor to the bioactive decapeptide in their early migratory phase, although some of them are fully committed to their morphological and biochemical phenotype (Livne et al. 1992). Grafts, derived from the embryonic nasal septum containing the migratory population of LHRH cells, transplanted into the adult brain, are capable of producing their fully processed neuropeptide product and recognizing their proper target in the host brain (Livne et al. 1992).

Table IV
Day of appearance of RH- and IH-RH perikarya in the hypothalamus and in extrahypothalamic brain areas of mammalian embryos

Peptide	Species	Day of hypothalamic appearance	Hypothalamic localization	Day of extrahypothalamic appearance	Extrahypothalamic localization	References
LHRH	rat	17	preoptic area	19	around the medial forebrain bundle septum	Horváth et al. (1982)
		2nd post-natal day	mediobasal hypothalamic area	2nd post-natal day		Daikoku et al. (1978)
		20.5	median eminence			
		17.5	lateral hypothalamic area	16.5	forebrain	Daikoku et al. (1984b)
		19	median eminence	15	olfactory bulb	Jennes (1989)
		20	median eminence	20	OVLT	Watanabe (1980)
	mouse	17	median eminence	17 11.5	OVLT olfactory placode	Gross and Baker (1977) Schwanzel-Fukuda and Pfaff (1989) Wray et al. (1989)
	guinea pig	last week of gestation	anterior mediobasal hypothalamus, preoptic area	last week of gestation	septum OVLT	Barry and Dubois (1974)
CRF	human	9th week	preoptic, pre-mammillary region			Paulin et al. (1977)
	rat	16.5	paraventricular nucleus			Daikoku et al. (1984a)
		newborn	paraventricular nucleus			Bugnon et al. (1982c)
THR	rat	16.5	dorsomedial nucleus,			Nishiyama et al. (1983)
		17.5	paraventricular nucleus			
		22.5	median eminence			
GHRH	rat	18.5	arcuate nucleus			Chikamori-Aoyama et al. (1986)
		18	mediobasal hypothalamus			Jansson et al. (1987)
		20	median eminence			Degennaro et al. (1986)
SRIF	rat	12.5	third ventricle floor	16.5	anterior forebrain	Daikoku et al. (1983, 1984)
		17.5	arcuate nucleus	17.5	medial septum	
			rostral hypothalamus		olfactory cortex	
		18.5	median eminence			Adachi et al. (1984)
				16	cerebellum	Inagaki et al. (1982)
	human	16th week				Bugnon et al. (1977b)
	mouse	19				Gross and Longer (1979)

The extracranial source of LHRH cells and their migratory capacity have evidently been proved both in avian and mammalian embryos; at present, however, no information is available whether migration of these peptidergic neurons might be considered a general rule valid also for other RHs and IHs. From a comparative point of view, the pattern of the early development of LHRH neurons suggests basic similarities between birds and mammals.

The time sequence concerning the first appearance of immunolabelled neurons in the chicken, regardless of their location was found to be as follows: LHRH appeared first, followed by TRH, SRIF and CRF (*see* Table III). More or less, a similar pattern could be traced also in mammalian embryos (*see* Table IV). The functional significance of the relatively early appearance of these peptides is still extensively studied.

A critical step in the development of the brain-pituitary-target organ axis is the initiation of tropic hormone synthesis in the embryonic pituitary. It has been shown in the developing rat pituitary that luteinizing hormone (LH) appears first in a few pituitary cells as early as day 16 or day 17 (Begeot et al. 1981, Nemeskéri et al. 1988). Factors initiating the first production of LH in the fetus are not clarified yet. Although LHRH regulates LH synthesis and release in adults, its possible role in the differentiation of the anterior pituitary is still controversial. The concept, that LHRH is not an inductive factor in the differentiation process of the primordial pituitary cells, is supported by several authors. *In vitro*, pituitary cells, obtained from 11-day-old embryos, maintained in culture were able to produce LH in the absence of LHRH (Watanabe 1987, Nemeskéri and Halász 1989). While co-culturing the developing diencephalons caused a marked increase in the size of the pituitary, the chronology of the onset of pituitary hormone production was not affected (Daikoku et al. 1982). Although these studies suggest that LHRH may not be mandatory for the initiation of gonadotropin synthesis, other data have provided evidence suggesting that LHRH has the ability to advance LH and FSH expression (Horacek et al. 1989). If LHRH is involved in advancing the first gonadotropin expression, 3 criteria must be met: 1) LHRH must be present in this early time of development, 2) it must be able to reach the developing pituitary and 3) it must bind to specific membrane receptors on the developing pituitary cells. Most data agree that the fetal gonadotropes are differentiated on day 16 of the rat fetus, which is the first day of LH appearance (Watanabe and Daikoku 1979, Begeot et al. 1981, Aubert et al. 1985, Nemeskéri et al. 1988). In contrast, other data suggest that the gonadotropes are differentiated earlier than assessed by the first appearance of LH, because the expression of receptors is already an indication of cell determination. Since LHRH receptors are present already on day 13 (Jennes 1990), it is suggested that these cells are determined to be gonadotropes at an earlier age. In conclusion, most data indicate that LHRH might be a factor determining the onset of LH synthesis, but it does not have a role in differentiation of the primordial pituitary cells.

Although the development of the functional relationship of the hypothalamo-hypophyseal-gonadal axis has been quite thoroughly studied, relatively few data are concerned with other systems. TSH has been detected earlier than TRH in our studies, but a reverse sequence was reported by Thommes et al. (1983, 1985); ACTH production precedes CRF production or appears simultaneously both in birds and mammals. GH seems to appear also before, or simultaneously with, the corresponding RH (GHRH) and IH (SRIF). To evaluate these data, the factors, described above in relation to the LHRH system, should also be taken into account.

Relatively wide variations of data were described by the different authors reporting the first day of appearance of a given RH- or IH-IR material even in the same mammalian species (e.g. rat). Therefore, it would be arbitrary to make unequivocal comparisons between birds and mammals in this respect. It is not the intent of this short monograph to provide detailed comparison between the different mammalian species.

It could be argued justifiably why only the appearance of the RH- or IH-IR perikarya has been taken into account in the present work, with less attention being paid to the IR fibres and terminals. Since numerous factors should be taken into account (species, localization within or outside the hypothalamus, sequential appearance of the different RHs or IHs, etc.) we tried to simplify the analysis somewhat artificially. Since neuropeptides are synthesized in the cell bodies of the neurons and are only transported by the axons, we chose to draw the conclusions of this chapter mainly on the basis of the developmental pattern of the RH- and IH-IR perikarya.

Co-existence of Two or More Releasing Hormones within the Same Nucleus or in the Same Neuron of the Brain

Our investigations, dealing with the localization of the different RH- or IH-IR neuron systems, established the occurrence of different neuropeptides in the same brain areas. It became evident that several hypothalamic and extrahypothalamic nuclei or areas are implicated in the different RH- or IH systems. The question was logically raised whether these brain regions contain neurons exhibiting different immunostaining properties or a co-localization of different neuropeptides exists. In other words, whether the cell population of such an area is a mosaic of neurons producing different RHs or the different RHs are co-expressed in one and the same neuron.

To approach this problem, the distribution of perikarya containing different neuropeptides is shown in the schematic sections of the avian brain (*Fig. 28*). The distribution of IR fibres and terminals immunostained for different neuropeptides is demonstrated in *Fig. 29*. Both schematic representations indicate that several well-defined hypothalamic and extrahypothalamic brain areas contain neurons showing different immunostaining properties. The majority of TRH- and CRF-IR perikarya are located in the paraventricular nucleus of the hypothalamus. All the three investigated RHs are present in neuronal cell bodies in the preoptic region. Some extrahypothalamic areas, such as the central gray of the midbrain and the accumbens nucleus, also contain two RHs (TRH and CRF). As indicated in *Fig. 29*, IR fibres and terminals of different RH systems are also present frequently in the same brain area. Dense plexus of TRH- and CRF-IR fibres were found in the region of the paraventricular hypothalamic nucleus. Great numbers of both CRF- and LHRH-IR fibres are present in different parts of the olfactory lobe. All three RHs are present in fibres within the hypothalamus, especially in the ventromedial nucleus and the periventricular hypothalamic area as well as in extrahypothalamic sites, mainly in the septum, the accumbens nucleus and the mesencephalic central gray (Mess and Józsa 1989).

Hypothalamic and extrahypothalamic brain regions, representing double or triple immunostaining properties regardless of the intraneuronal localization of

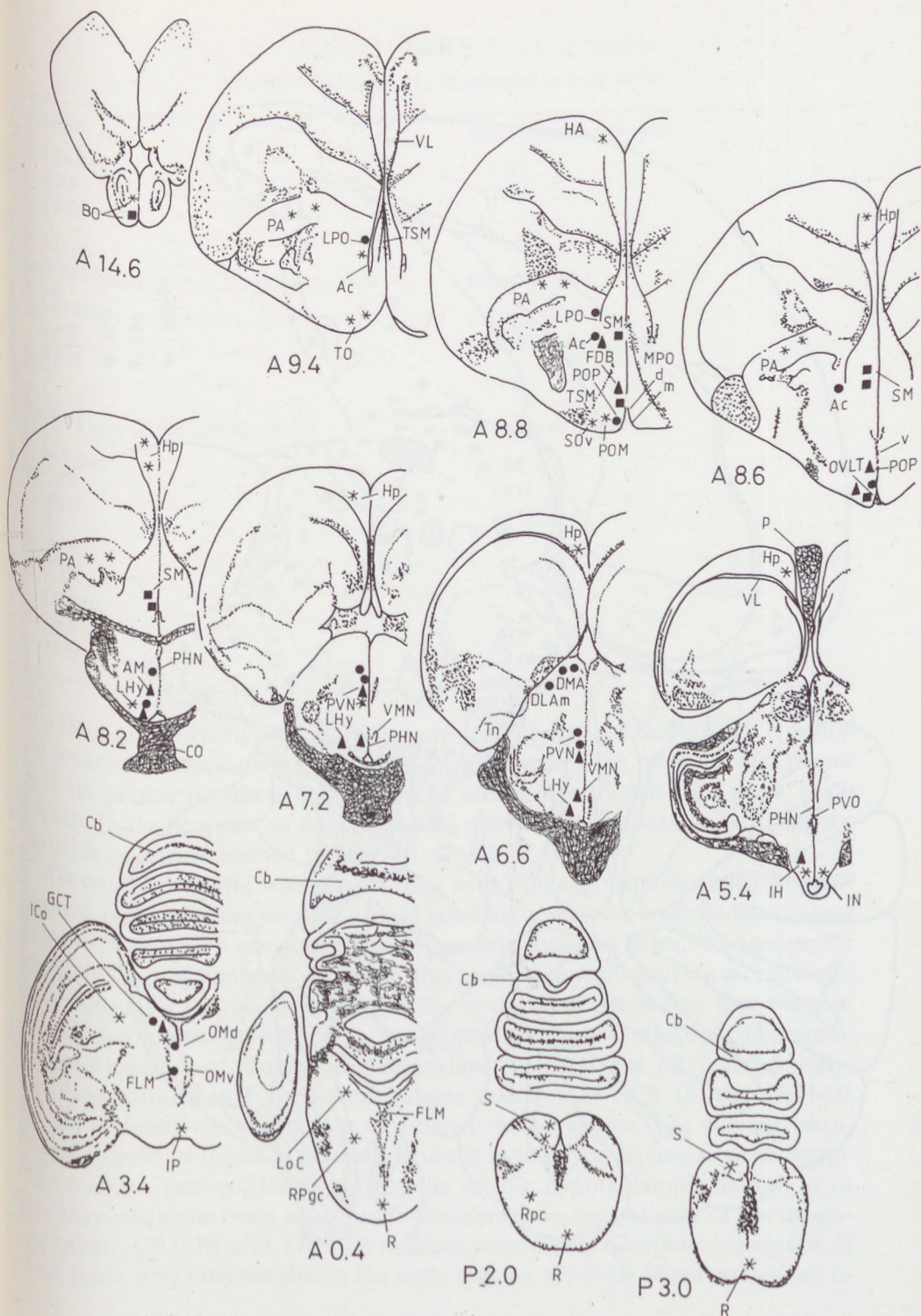


Fig. 28. Diagrammatic illustration of the location of different RH- and IH-IR cell bodies in the central nervous system of the avian brain. The schemes are based on the atlas of Kuenzel and Masson (1988). Perikarya, immunoreactive to LHRH (squares), CRF (circles), TRH (triangles) and SRIF (asterisks) are represented, respectively. For abbreviations see list on pp. 11–12

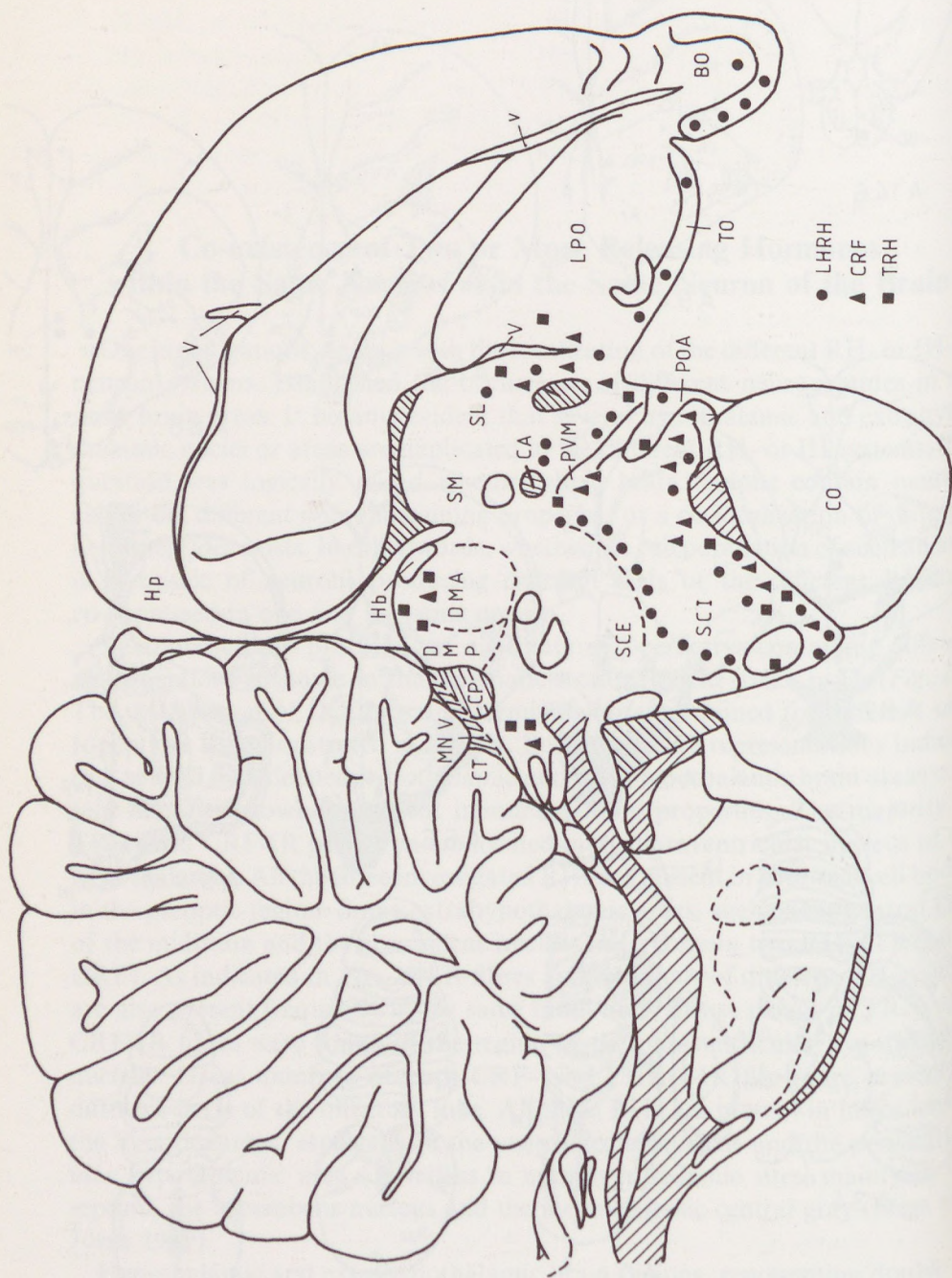


Fig. 29. Schematic drawing illustrating the distribution of different RHR-IR fibres in the chicken brain. Fibres, immunoreactive to LHRH (dots), CRF (triangles) and TRH (squares) are illustrated in a sagittal scheme. For abbreviations see list on pp. 11-12.

Table V

Presence of different RH-IR material in brain areas

Preoptic area	Paraventricular nucleus
LH-RH	CRF
CRF	TRH
TRH	
Subependymal layer of the third ventricle	Olfactory lobe
LH-RH	LH-RH
CRF	CRF
TRH	
Central gray of the mesencephalon	Accumbens nucleus
LH-RH	CRF
CRF	TRH
TRH	
Septum	
LH-RH	
CRF	
TRH	

the IR material (perikarya, fibres or terminals) are listed in *Table V*. It would be somewhat speculative at the moment to interpret the presence of different RH-IR perikarya, fibres and terminals being intermingled in a given brain region. In the next part of this chapter we return to this question discussing the possible pathways formed by the RH- or IH-IR neurons.

To establish clearly whether neurons with similar morphology actually co-express more peptides or not, a dual labelling immunocytochemical technique was performed. We are aware that arguments could be presented against the multiple staining methods. However, this method can still provide morphological evidence for the real probability of one or the other alternative. Our observations, based on this technique, rather support the "mosaic theory". The simultaneous presence of neurons immunostained for CRF and TRH, respectively, are demonstrated in *Fig. 30*. Simultaneous detection of CRF-IR and TRH-IR neural elements was carried out in different brain regions. The different chromogens could be found in separate neurons in the hypothalamus (paraventricular nucleus, periventricular part of the middle hypothalamus) as well as in extrahypothalamic brain areas (accumbens nucleus, central gray of the mesencephalon). CRF-IR and TRH-IR neurons were evenly distributed; neurons of both types were intermingled in the same regions. CRF-IR fibres juxtaposed to

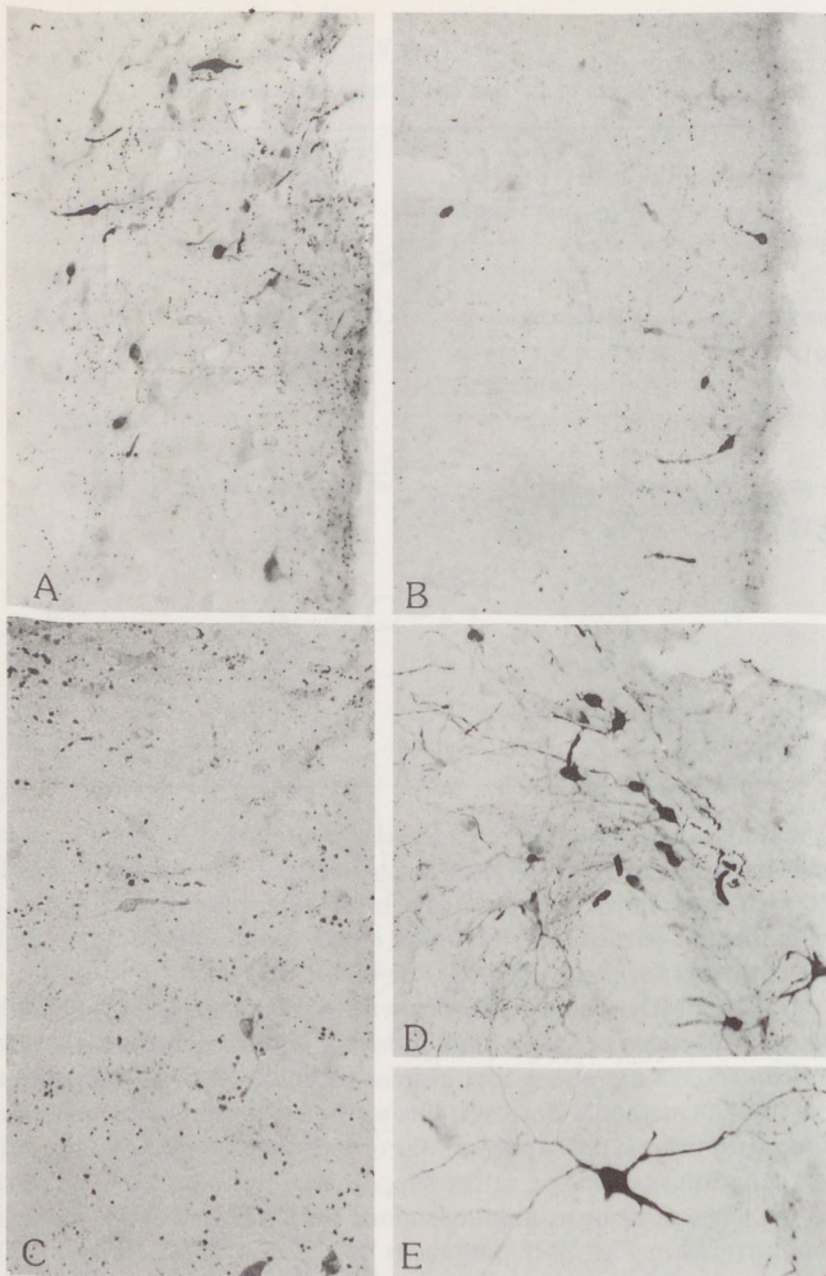


Fig. 30. Double immunostained preparations showing the distribution of the silver-gold intensified CRF-IR neurons (black) and the non-intensified TRH-IR neurons (pale). *A)* Both types of IR cells are evenly distributed in the periventricular and medial subdivisions of the paraventricular nucleus. $\times 180$; *B)* Separate parvocellular neurons containing either CRF- or TRH-IR material are found in the periventricular area of the middle hypothalamus in close proximity to the ependymal wall of the third ventricle. $\times 180$; *C)* Weakly labelled TRH-IR neurons (pale) are surrounded by numerous CRF-IR fibres in the region of the medial preoptic nucleus. $\times 180$; *D)* Distribution of CRF- and TRH-IR structures in extrahypothalamic location, in the region of the accumbens nucleus. Note the numerous intensified CRF-IR neurons and the few, weakly immunostained TRH-IR neurons. $\times 180$; *E)* High power magnification showing a "Golgi-like" appearance of a multipolar silver-gold intensified CRF-IR neuron observed in the accumbens nucleus. $\times 400$

TRH-IR perikaryon or processes were frequently observed. The present preliminary study suggests the possibility of neural interactions between CRF-IR and TRH-IR neurons in the chicken central nervous system. Further ultrastructural studies are required to elucidate the synaptic connections between these peptides. We failed to find co-expression of CRF- and TRH-immunoreactivity within one and the same neuron. This is in good agreement with the findings of Merchenthaler et al. (1982) obtained on the rat in our laboratory. He described CRF- and arginine vasopressin-IR cells in the paraventricular hypothalamic nucleus, but the different IR materials were always found in separate neurons.

On the other hand, several reports indicate the existence of the intraneuronal co-localization of two or more neuropeptides. Meister et al. (1988) demonstrated two types of immunopositive granules (CRF and glutamic acid decarboxylase, GAD; CRF and gamma-aminobutyric acid, GABA) within the same perikaryon of the paraventricular hypothalamic nucleus in the albino rat. Similarly, co-localization of GRF and neuropeptide Y (NPY) immunopositivity was also described in the neurons of the infundibular area of the human brain (Ciofi et al. 1988). In our study, we failed to find evidence indicating the intraneuronal co-localization of two RHs. According to the above-mentioned reports, a neuron is able to synthesize two or more neuropeptides simultaneously. One can speculate whether it is a fundamental principle in the CNS that in a neuron a RH or an IH can be co-expressed with more neurotransmitters (e.g. neuropeptide-Y, GAD, GABA), but not with more RHs or IHs. In fact, intraneuronal co-localization of two or more classical RHs or IHs have not yet been demonstrated.

Axonal Projections of the Hypothalamic and Extrahypothalamic RH-IR Perikarya and their Functional Role

According to the original concept of Harris (1955), the hypothalamus acts as the key mediator between the central nervous system and the pituitary gland, effecting a wide range of responses to both physical and psychological stimuli. The RHs and IHs (as indicated in their names) were considered neuropeptides of hypothalamic origin regulating the tropic hormone secretion and release in the anterior pituitary. Based on this concept, the close relationship between the axon terminals of the RH- and IH neurons and the capillaries in the median eminence is a prerequisite for these neuropeptides to enter the portal circulation, and to act as neurohormones.

Since many extrahypothalamic regions contain different types of RH- or IH-IR perikarya, this concept should have been modified or supplemented. As early as 1977, Korányi et al. established that TRH, LHRH and SRIF play a

role in the normal activity of the brainstem reticular formation and the hippocampus in the female rat. The neurotransmitter function of the RHs and IHs was also confirmed in birds by several laboratories (Krisch 1977, Blähser 1978, Johansson et al. 1984).

On grounds of the bifunctional role of RHs and IHs one can assume the existence of two, more or less separated populations of the RH- or IH neuron systems: neurons, related to the hypothalamo-pituitary system, playing the role of stimulation or inhibition of trop-hormone secretion according to the traditional concept, and extrahypothalamic RH- or IH neuron systems where RHs and IHs act as neurotransmitters or neuromodulators. This latter system might influence the functional activity of the hypothalamo-pituitary axis, but it might have a neurotransmitter function in a more general sense not related to the neurohormonal RH- or IH systems. We attempt to summarize our concept as

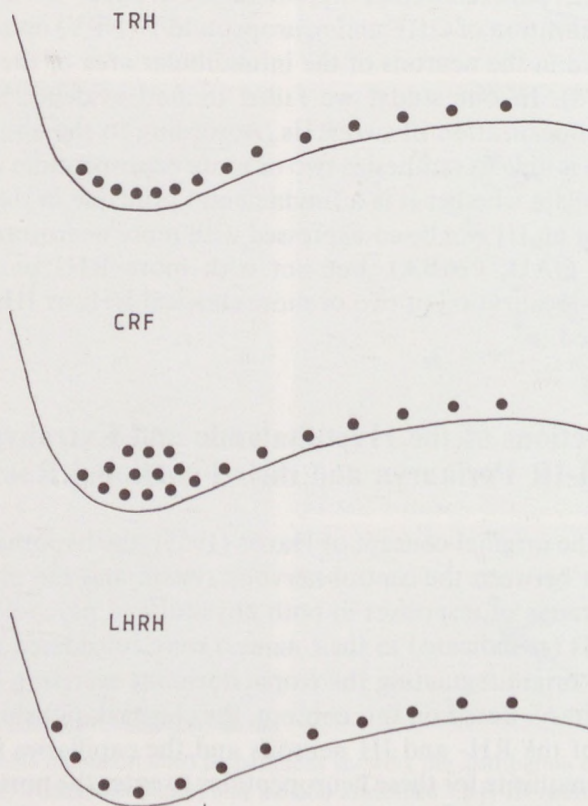


Fig. 31. Schematic sagittal drawing of the median eminence of birds illustrating the distributional pattern and density of RH-IR fibres and terminals. CRF-IR fibres appear to be more numerous in the anterior median eminence when compared to TRH. Both CRF and TRH fibres appear to be more numerous as compared to LHRH

outlined above, adding that this concept is not a mere speculation. Preliminary data are available indicating the existence of the different types of proposed neuronal connections. However, the verification of this concept needs further, more systematic tract-tracing investigations, as planned in our future project.

The vast majority of the hypothalamic RH-IR neurons project to the ME. All the three types of investigated RH-IR terminals are present in the external zone of the ME. LHRH-IR fibres and terminals are evenly distributed in the anterior and posterior parts of the ME, while TRH and CRF-IR axons and nerve endings were predominantly found in the anterior division (*Fig. 31*). This is in accordance with our previous observations (Józsa et al. 1979) that LH-IR cells are located both in the cephalic and caudal lobes of the avian pituitary, while most of the TSH- and ACTH-IR cells are concentrated solely in the cephalic lobe. In conclusion, RH-IR axons terminating in the anterior division of the ME are involved in the regulation of the cephalic lobe of the pituitary, while nerve endings found in the posterior division of the ME play a role in the control of the caudal lobe of the pituitary.

The RH- or IH-IR fibres of hypothalamic origin running to the ME do not usually form well-defined larger tracts. They run toward the ME forming smaller fascicles, or as scattered fibres. We failed to find direct projections of the extrahypothalamic RH- or IH-IR perikarya to the ME, with the exception of neurons located in the septum. LHRH fibres, originating from the septum being intermingled with axons of preoptic LHRH perikarya, form the median preoptico-infundibular LHRH tract above the optic chiasm towards the ME.

Hypothalamic RH- or IH-IR neurons serve also for intrahypothalamic connections (*Fig. 32, neuron 2*) and/or project to extrahypothalamic brain areas (*Fig. 32, neuron 3*). The RH- or IH-IR fibres of extrahypothalamic origin project either to different IR- or immunonegative neurons of the hypothalamus (*Fig. 32, neuron 4*), or to remote extrahypothalamic regions (*Fig. 32, neuron 5*).

In *Fig. 33* we attempt to provide a more detailed analysis of the possible connections and functional role of the hypothalamic RH- or IH-IR neurons. The majority of IR cells terminate—as mentioned above—on portal capillaries of the ME, regulating the production and release of pituitary tropic hormones (*neuron 1*). It can be assumed that some RH- or IH-IR axons of hypothalamic origin, running towards the ME, might have axon collaterals projecting to extrahypothalamic neurons (*neuron 2*). Other hypothalamic IR neurons might terminate on another IR neuron that projects to the ME (*neuron 4*). As it has been postulated, hypothalamic hormones could regulate their own secretion by means of ultrashort loop feedback mechanism. On the other hand, this latter type of neuronal connections might also influence the hypothalamic release of other types of RHs or IHs as well. Another possible variant of connections can be that the hypothalamic RH- or IH-IR cells are involved in hypothalamo-extrahypothalamic neuronal interactions (*neuron 3*). These axons spread to

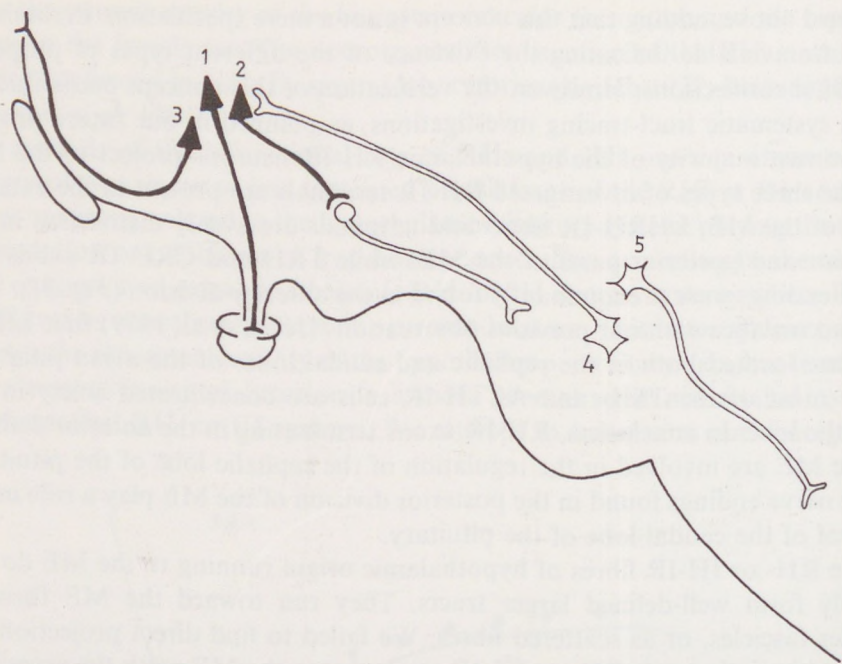


Fig. 32. Possible variations of projections of RH-IR neurons on a schematic sagittal drawing. 1) RH-IR neurons of the hypothalamus projecting to the median eminence; 2) RH-IR neurons of the hypothalamus terminating on immunonegative hypothalamic neurons; 3) RH-IR neurons of the hypothalamus projecting to extrahypothalamic brain regions (e.g. to the telencephalon); 4) RH-IR neurons of the brainstem projecting to hypothalamic RH-IR neurons; 5) RH-IR neurons of the brainstem as a source of descending fibres

different, mainly immunonegative, structures of the limbic system, the brainstem or even of the spinal cord. These projections might play a role in the regulation of behaviour related to endocrine functions. For instance, LHRH terminals detected around large immunonegative neurons of the nucleus of the tectal commissure of the chicken, might influence sexual behaviour.

Regarding the large variety of fibre projections of the different hypothalamic and extrahypothalamic RH- or IH-IR neurons, and also considering the differences in the ontogenetic development of the hypothalamic and extrahypothalamic IR systems, the following conclusion could be drawn: RH- or IH neuron systems, sending their axons to the portal vessels of the ME are considered RH- or IH systems in the classical sense of their individual designations. All of the RH- or IH-IR neurons terminating elsewhere in the CNS, except the ME (predominantly the extrahypothalamic IR neurons belong to this latter system), are involved in extrahypophyseal actions. In this case RHs or IHs play a role as neurotransmitters or neuromodulators. This latter neuronal activity might well be indirectly related to the function of the corresponding RH-tropic hormone-target axis.

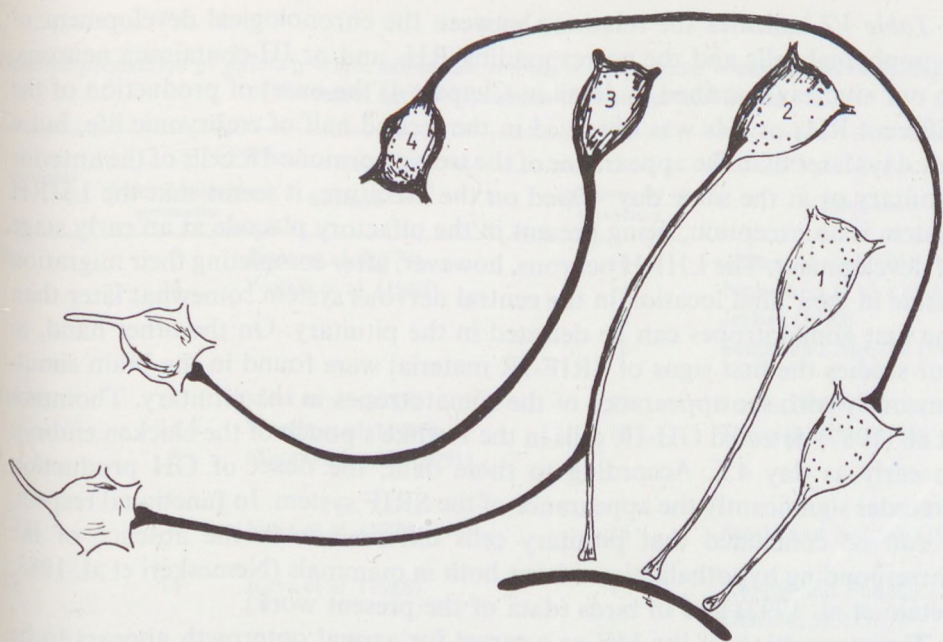


Fig. 33. Possible connections of RH-IR neurons of hypothalamic origin (triangular cell). 1) RH-producing cell projects to the median eminence; 2) RH-producing cell of the hypothalamus projects to the median eminence and gives collaterals to extrahypothalamic neurons (polygonal cell); 3) RH-producing cell of the hypothalamus terminates on extrahypothalamic neuron (polygonal cell); 4) RH-producing cell of the hypothalamus (dark triangular cell) terminates on other hypothalamic neuron (light triangular cell)

Sequential Relationship between the Onset of Production of the Releasing and Inhibiting Hormones in the Brain and the Onset of Production of the Pituitary Tropic Hormones

About twenty years ago, we demonstrated in chicken embryos that the hormone secretion of the thyroid gland and the adrenal cortex initiates independently before the onset of the production of the corresponding pituitary tropic hormones (Mess and Straznicky 1970). The results of these studies, performed with the aid of hormone bioassays and different physiological and morphological techniques, were completed later with immunocytochemical investigations. We have shown (Józsa et al. 1979) that tropic hormone-IR cells appear in the developing pituitary of the chicken embryo during the first half of embryonic life, but always a few days later than the hormone production of the corresponding target gland.

Table VI indicates the relations between the chronological development of hypophyseal cells and the corresponding RH- and/or IH-containing neurons. In our studies (described in detail in Chapter 4) the onset of production of the different RHs or IHs was observed in the second half of embryonic life, but a few days later than the appearance of the tropic hormone-IR cells of the anterior pituitary or at the same day. Based on the literature, it seems that the LHRH system is an exception, being present in the olfactory placode at an early stage of development. The LHRH neurons, however, after completing their migration reside in their final location in the central nervous system somewhat later than the first gonadotropes can be detected in the pituitary. On the other hand, in our studies the first signs of SRIF-IR material were found in the brain simultaneously with the appearance of the somatotropes in the pituitary. Thommes et al. (1987) detected GH-IR cells in the Rathke's pouch of the chicken embryo as early as day 4.5. According to these data, the onset of GH production precedes significantly the appearance of the SRIF system. In functional respect, it can be concluded that pituitary cells differentiate in the absence of the corresponding hypothalamic neurons both in mammals (Nemeskéri et al. 1988, Sétáló et al. 1992) and in birds (data of the present work).

The recognition of the ME as a target for axonal outgrowth appears to be an intrinsic property of the RH cells, but it is unlikely that these hormones can reach the pituitary primordium through the bloodstream at an early stage of development. Thus, the functional unit of the hypothalamo-hypophyseal system cannot be established before the RHs and IHs are transported to the developing pituitary and bind to specific receptors. In the chicken embryo, the definitive vascular connection, e.g. the portal vascular plexus, is present by day 12 (Thommes and Russo 1959). In mammals, Dubois and Hemming (1987) reported that the hypothalamo-hypophyseal portal system is complete during the early fetal life in some mammalian species. These authors pointed out that factors implicated in the regulation of the pituitary hormone secretion are generally the same in the fetus as in the adult, but the intensity of response of the embryonic pituitary cells is variable depending on species differences (Dubois and Hemming 1991). To the contrary, Mitskevich (1990) stated that the hypothalamic neurohormones reach the pituitary only at the end of the prenatal period in mammals.

Another crucial point is the development of the specific receptors. Heidet et al. (1990) detected SRIF binding sites (receptors) in the pituitary of the mouse after birth only (1st postnatal day), although SRIF is present earlier in the embryonic brain. In contrast, Jennes (1990) has shown LHRH receptors on day 13 in the pituitary of the rat, 3 days before the onset of LH production. Since our work was not focussed on the investigation of receptor-ontogeny, further discussion of this topic is omitted.

Table VI

Onset of production of pituitary tropic hormones and the corresponding releasing and inhibiting hormones of the central nervous system in the chicken and the rat

Hormones	Chicken		Rat	
	Embryonic day of appearance	References	Embryonic day of appearance	References
LH	7	Józsa et al. (1979)	16	Aubert et al. (1985)
	4	Woods et al. (1985)	16	Nemeskéri et al. (1988)
			16	Jennes (1990)
			18	Sétáló and Nakane (1976)
LHRH	18	Mess et al. (1983)	15	Jensen (1989)
	5.5	Woods et al. (1985)	18	Horváth et al. (1982)
	3	Murakami et al. (1991)		
ACTH	7	Józsa et al. (1979)	16	Sétáló and Nakane (1976)
CRF	14	Józsa et al. (1986)	15	Rundle and Funder (1988)
			17	Grino et al. (1989)
			18	Bugnon et al. (1984)
TSH	6.5	Thommes et al. (1983)	17	Sétáló and Nakane (1976)
	7	Józsa et al. (1979)		
TRH	14	Józsa et al. (1989)	19	Schaeffer and Brownstein (1980)
	4.5	Thommes et al. (1985)		
GH	4.5	Thommes et al. (1987)	17	Martinoli and Pelletire (1990)
	12	Józsa et al. (1979)	18	Watanabe and Daikoku (1979)
			18	Smets et al. (1989)
			19	Sétáló and Nakane (1976)
GHRH			18	Jansson et al. (1987)
			20	Degennaro et al. (1986)
SRIF	12	Mess et al. (1983)	20	Shiosaka et al. (1982)
			day of birth	Horváth et al. (1982)

Comparing the development of the different levels of the hypothalamo-pituitary-target organ systems, the following conclusions can be made:

The target glands differentiate in the absence of the tropic hormones and are capable of hormone production to a certain level without tropic hormone stimulation. Similarly, the adenohypophyseal cells differentiate and are able to produce tropic hormones before receiving hypothalamic modulation by the

RHs or IHs. Although it appears evident that RHs do not affect the determination of prospective tropic cells, these peptides may have the ability to advance the first expression of tropic hormones (for instance, when the appearance of an RH precedes, or coincides with, the onset of the corresponding tropic hormone production). The functional hierarchy between the different levels of the neuroendocrine system being characteristic in the adults, however, appears to develop around the late embryonic to early posthatching period.

SUMMARY

1. The anterior parts of the hypothalamus, especially the anterior periventricular regions and the paraventricular nucleus, and the preoptic area are the most active parts of the hypothalamus in the production of the different RHs and IHs in birds. The majority of the RH- and IH-IR perikarya is located in these areas. Scattered cells or smaller cell groups can also be found in the posterior part of the hypothalamus (ventromedial, posterolateral, premammillary regions).

2. The regions of the limbic system (septum, olfactory bulb and lobe) and the brainstem (mesencephalic central gray, pontine tegmentum) appear to be the richest extrahypothalamic regions containing the greatest number of RH- or IH-IR perikarya. Scattered IR cells or smaller cell groups of IR neurons are present nearly throughout the entire CNS.

3. High concentrations of the RH-IR axons accumulate in the external zone of the ME. TRH- and CRF-IR neurons project predominantly to the anterior division of the ME, while LHRH terminals are evenly distributed throughout the entire length of the external zone in both the anterior and posterior divisions of the ME.

4. Some hypothalamic RH- and IH-IR axons establish synaptic junctions with intrahypothalamic neurons immunoreactive for the same peptide and/or with non-immunoreactive neural elements. Some intrahypothalamic IR neurons project to extrahypothalamic brain regions.

5. RH- or IH-IR neurons located outside the hypothalamus may have projections to IR or immunonegative hypothalamic neurons or to more remote extrahypothalamic structures.

6. In some hypothalamic areas and nuclei (paraventricular nucleus, anterior periventricular area) and even in some extrahypothalamic sites (accumbens nucleus, olfactory lobe, septum, mesencephalic central gray) different types of RH- or IH-IR perikarya are located simultaneously. Whether this is due to juxtaposition of different IR neural elements or to intraneuronal co-localization of different IR granules, still remains an open question. Our present results speak in favour of the former possibility.

7. All RH- and IH-IR systems are already present during the course of embryonic development in the investigated avian species. The LHRH system appears first (olfactory placode), followed by TRH-, SRIF and CRF systems somewhat later.

8. CRF immunoreactivity can be detected first in the hypothalamus and somewhat later in extrahypothalamic sites. In contrast, SRIF-IR material appears first in extrahypothalamic location. Since LHRH neurons migrate from an extracerebral site toward the brain, these cells reside in their final distribution regions, in the hypothalamus and the extrahypothalamic brain regions, nearly at the same time.

9. The appearance of the first sign of pituitary tropic hormones in most cases precedes the appearance of the corresponding RH-IR or IH-IR neural elements in the brain, indicating that the differentiation process of the pituitary cells takes place at an early stage in the absence of hypothalamic influence. Reverse time sequence may also be the case, suggesting that the RHs might be inductive factors in the onset of the pituitary hormone production. The hypothalamo-pituitary systems characteristic of the adult stages, however, become operative after the portal vascular connection is established and after the onset of expression of specific receptors.

10. On grounds of the local distribution and possible neuronal projections, as well as of the different developmental patterns, the authors propose to subdivide the RH- and IH neuronal systems into two main functional groups: the hypophyseotropic system, including those RH- and IH-containing neurons of different brain regions that project to the median eminence and the hypothalamic and extrahypothalamic RH- and IH neuronal systems that serve for interneural connections. In the latter systems RHs and IHs are considered neurotransmitters or neuromodulators.

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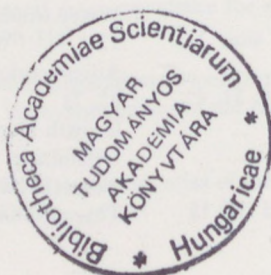
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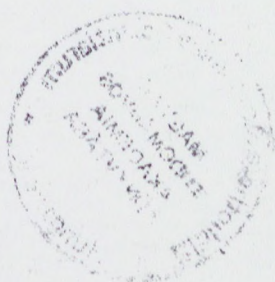
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This book summarizes the results of an immunocytochemical research work of nearly 15 years. The releasing and inhibiting hormone-producing neuron systems are described in birds (chicken) and compared with mammals (rat) from comparative-endocrinological and developmental-endocrinological points of view. Plates of microphotographs and schemes of the different brain areas make this work a kind of histological atlas of the avian central nervous system. Comprehensive tables assist the reader in orientation.

This volume is a useful source for all those involved with the research in biology, medicine, veterinary medicine and animal husbandry, or even for experts in agriculture with an interest in poultry farming.

