# Hypothalamic monoaminergic systems in ontogenesis: development and functional significance

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**ABSTRACT This paper summarizes recent data on the development of hypothalamic monoaminergic (MArgic) systems which play a key role in neuroendocrine regulation in adult mammals. Hypothalamic catecholaminergic (CArgic) and 5-hydroxytryptamine (=serotonin, 5-HT) systems undergo synchronous development that begins from the origin of dopaminergic neurons in the hypothalamus and 5-HT neurons in the *raphe nucleus* long before birth. Moreover, some hypothalamic neurons of fetuses and young rats partly express the 5-HT phenotype patterns: the 5-HT specific uptake and synthesis from 5-hydroxytryptophan. Differentiation of MArgic neurons is manifested in expression of specific enzymes and MA synthesis as well as in the onset of the MA specific uptake and potassium-stimulated release. In the course of differentiation, MArgic neurons send their axons to target neurons, *hypophysial* portal circulation, the third ventricle and adenohypophysis, followed by the establishment of specialized contacts: synapses, axo-vascular, axo-ventricular and axo-glandular. The hypothalamic CAs and 5-HT firstly appeared in embryogenesis control phenotype expression of target neurons: gene expression, specific synthesis, uptake and release, axonal growing, etc. In turn, the development of the hypothalamic MArgic systems is under control of MAs (autoregulation) and hormones of the peripheral endocrine glands (androgens). Conversely, there is a minor role, if any, of the maternal and placental neurohumoral factors in differentiation of MArgic neurons.**

KEY WORDS: *development, hypothalamus, neurons, catecholamines, serotonin*

## Introduction

Since the first applying of the histofluorescent technique for detection of monoamines (MAs) (Falck *et al.*, 1962), it has been repeatedly demonstrated that the hypothalamus contains three major populations of dopaminergic (DArgic) neurons located in the zona incerta, periventricular and arcuate nuclei (Björklund and Lindvall, 1984). In addition to DArgic axons, the hypothalamus receives noradrenergic and adrenergic afferents from the brainstem as well as 5-hydroxytryptamine (=serotonin, 5-HT) axons from the mesencephalic raphe nucleus (Fig.1) (Steinbusch and Nieuwenhuys, 1981). In adults, hypothalamic MAs are involved in neuroendocrine regulations playing a role of: a) neurotransmitters controlling the secretory activity of the hypothalamic neurons, b) neuromodulators regulating the release of neurohormones from adjacent axons in the median eminence and of hormone secretion from adenohypophysial cells, c) neurohormones delivered via the hypothalamo-hypophysial portal circulation to the target adenohypophysial cells (Fig. 1) (Weiner *et al.*, 1988; Everitt *et al.*, 1992). In addition to the activating reversible effects, MAs provide morphogenetic irreversible actions on the targets during critical periods of ontogenesis (Lauder, 1993).

This paper summarizes the data on the development of the hypothalamic MArgic systems and on the role of MAs in differentiation of the targets obtained over last ten years by the Laboratory of Hormonal Regulations (Moscow, Russia) in collaboration with Russian and Western partners (see Acknowledgements, References).

## Development of the hypothalamic monoaminergic systems

### *Dopaminergic neurons*

Immunocytochemistry of tyrosine hydroxylase (TH), the first enzyme of CA synthesis, is among the most suitable approaches for studying the differentiation of CArgic neurons and their fiber projec-

*Abbreviations used in this paper:* AADC, aromatic L-amino acid decarboxylase; CA, catecholamine; DA, dopamine; E, embryonic day; 5-HT, 5-hydroxytryptamine (serotonin); IR, immunoreactive; MA, monoamine; P, postnatal day; TH, tyrosine hydroxylase.

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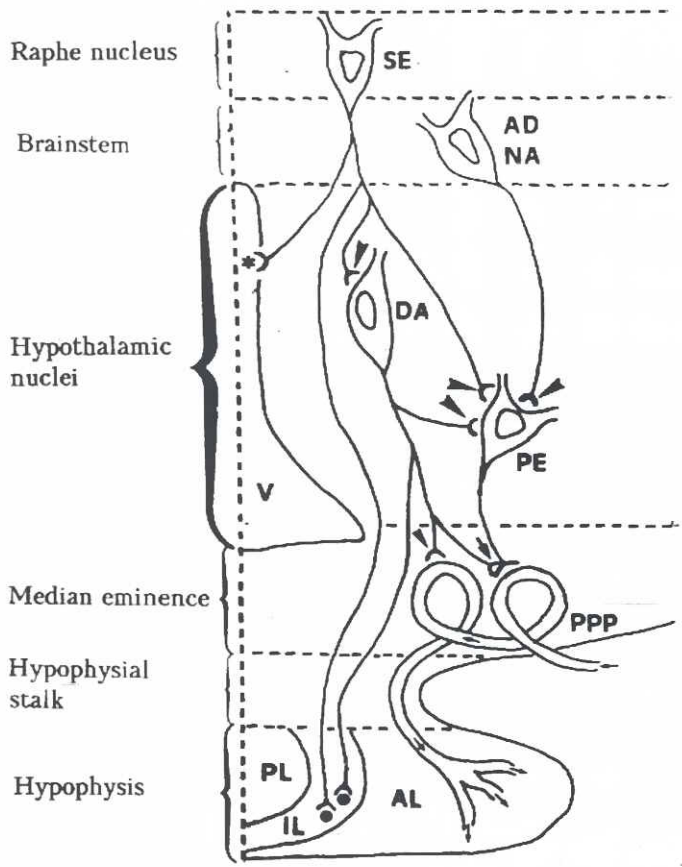


**Fig. 1. Schematic representation of the hypothalamic monoaminergic systems.** *AD, adrenergic neuron; AL, anterior lobe; DA, dopaminergic neuron; IL, intermediate lobe; NA, noradrenergic neuron; PE, peptidergic neuron; PPP, primary portal plexus; SE, serotoninergic neuron; V, third ventricle. Contacts: star, axo-ventricular; black dot, axo-glandular; small arrowhead, axo-vascular; large arrowhead, axo-somatic and axo-dendritic; large arrow, axo-axonic. Small arrow, direction of the bloodstream in portal circulation.*

tions because the enzyme is expressed early in ontogenesis and distributed from perikarya to fiber terminals (Ugrumov *et al.*, 1989c,d). However, the differentiating hypothalamic TH-immunoreactive (IR) neurons might be considered as true DArgic neurons with caution because some of them lack aromatic L-amino acid decarboxylase (AADC), the second enzyme of the DA synthesis. In turn, TH does not present obligatory in the neurons, possessing AADC (Balan *et al.*, unpublished). Therefore, the double labeling of both enzymes should be used for identification of the true DArgic neurons.

It is generally accepted that the neuron differentiation begins just after the cessation of proliferation of the ventricular cell-precursor. «Long-survival» [$^3$H]thymidine autoradiography combined with TH immunocytochemistry was used to determine the birthdates of DArgic neurons in the zona incerta, periventricular and arcuate nuclei of male and female rats (Borisova *et al.*, 1993; Balan *et al.*, in press). In males, DArgic neurons originate in the zona incerta mainly from the 12th embryonic day (E; the day of conception being the 1st embryonic day) to E13, while in the periventricular and arcuate nuclei this process is prolonged till E16. The maximal yield of DArgic neurons corresponds to E12 in the zona incerta, to E12-E13 in the periventricular nucleus and to E15 in the arcuate nucleus. There is sexual dimorphism in schedule of the DArgic neuron origin in each hypothalamic region: a) in the zona incerta neuron generation in males precedes that in females while there is a reverse in the arcuate nucleus, b) general yield of the neuron production in any studied hypothalamic region of females exceeds that in males. These differences are considered as a manifestation of genetic sexual dimorphism (Balan et al., in press) because they are displayed before the onset of androgen secretion by male gonads at E16 (Baum *et al.*, 1991).

DArgic neurons undergo striking morphological modifications in the course of differentiation. The initial small oval neurons, observed at E13, possess one or two short unbranched processes, terminating with growth cones. Two days later, multipolar neurons appear, additionally (Fig. 2a). From E18 onwards, cell bodies increase in size, and their processes in length (Fig. 2b) (Ugrumov *et al.*, 1989c). After birth, three neuronal types are distinguished: a) small uni- and bipolar neurons with short and narrow unbranched processes, located mainly in the mediobasal hypothalamus (arcuate nucleus) (Fig. 2c); b) large multipolar neurons with long ramified processes, distributed dorsally in the zona incerta, the dorsomedial nucleus (Fig. 2c) and the paraventricular nucleus; c) rather large bipolar neurons, occupied the periventricular nucleus (Ugrumov *et al.*, 1989c). So far, only DArgic neurons of the suprachiasmatic (Ugrumov *et al.*, 1994a) and arcuate nuclei (Ugrumov *et al.*, unpublished), belonging to the first type, were studied at the electron microscopic level. Both in fetuses and postnatal rats, these neurons possess relatively large nuclei and scanty cytoplasm as well as poorly developed Golgi complex and granular endoplasmic reticulum. Still, over the perinatal period, their size slightly increases, the nuclei become indented, dense core vesicles appear in the area of the Golgi complex, showing the onset of the secretory activity. This coincides with the innervation of DArgic neurons by immunonegative axons (Figs. 3,4). The neurons remain unchanged from the second postnatal day (P2, the day of parturition being the first postnatal day) until puberty, suggesting the onset of their functioning as early as in newborns.

The functional maturation of DArgic neurons is directly related to expression of enzymes of DA synthesis and to the appearance of membrane mechanisms for DA uptake and release in response to the membrane depolarization. Although TH and AADC were first detected in hypothalamic neurons at E13-E15, only a small portion of the neurons express simultaneously both enzymes, thus, being capable of DA synthesis (Balan *et al.*, unpublished). In fact, these cells show CA fluorescence from E16 that coincides with the first detection of DA specific uptake and $K^+$-stimulated $Ca^{2+}$-dependent release (Borisova *et al.*, 1991). In general, these data show that from E16 DArgic neurons synthetize DA being capable to release it in response to the adequate physiological stimuli.

### *Serotoninergic neurons*

5-HT-IR neurons were first detected in the rat raphe nucleus at E12-E14 (Wallace and Lauder, 1992). They express 5-HT synthesis just after the last mitotic division of cell precursors (Lauder and Bloom, 1974), followed by their differentiation and axonal growing to the hypothalamus. The question on the existence of 5-HT neurons in the developing hypothalamus, still, remains opened.

First autoradiography showed that hypothalamic neurons in the suprachiasmatic and dorsomedial nuclei of fetal and young rats have an ability for [$^3$H]5-HT specific uptake (Ugrumov *et al.*, 1986). Conversely, immunocytochemistry failed to detect 5-HT-IR neurons in the intact perinatal rats (Ugrumov *et al.*, 1986), though these neurons become visible after the acute treatment of fetuses and early postnatal rats with L-tryptophan, the amino acid precursor of the 5-HT synthesis, and pargyline, an inhibitor of the monoamine oxidase activity (Fig. 5) (Ugrumov *et al.*, 1988; 1989b).

Two populations of 5-HT-IR neurons were detected in fetuses and infants: in the dorsomedial nucleus and anterolateral hypothalamus (Ugrumov *et al.*, 1989b). In fetuses both populations, while in neonates only the later, consist of the intensely immunostained bipolar elongated neurons with the long bifurcated processes (Figs. 5a,b). In young rats, the neurons of the dorsomedial nucleus weakly immunostained are oval in shape and small in size, having one or two short unbranched processes (Fig. 5c) (Ugrumov *et al.*, 1989b). 5-HT immunostaining provoked by treatment with L-tryptophan and pargyline was prevented by the preliminary infusion of fluoxetine, an inhibitor of 5-HT uptake. It means that immunostaining is explained rather by specific uptake of intercellular 5-HT than by its synthesis from L-tryptophan, catalyzed by tryptophan hydroxylase (Ugrumov *et al.*, 1989b). Still, decarboxylation of 5-hydroxytryptophan to 5-HT by AADC is rather probable since both neuronal populations in perinatal rats become 5-HT-IR following administration of pargyline and 5-hydroxytryptophan (Ugrumov *et al.*, 1988, 1989b).

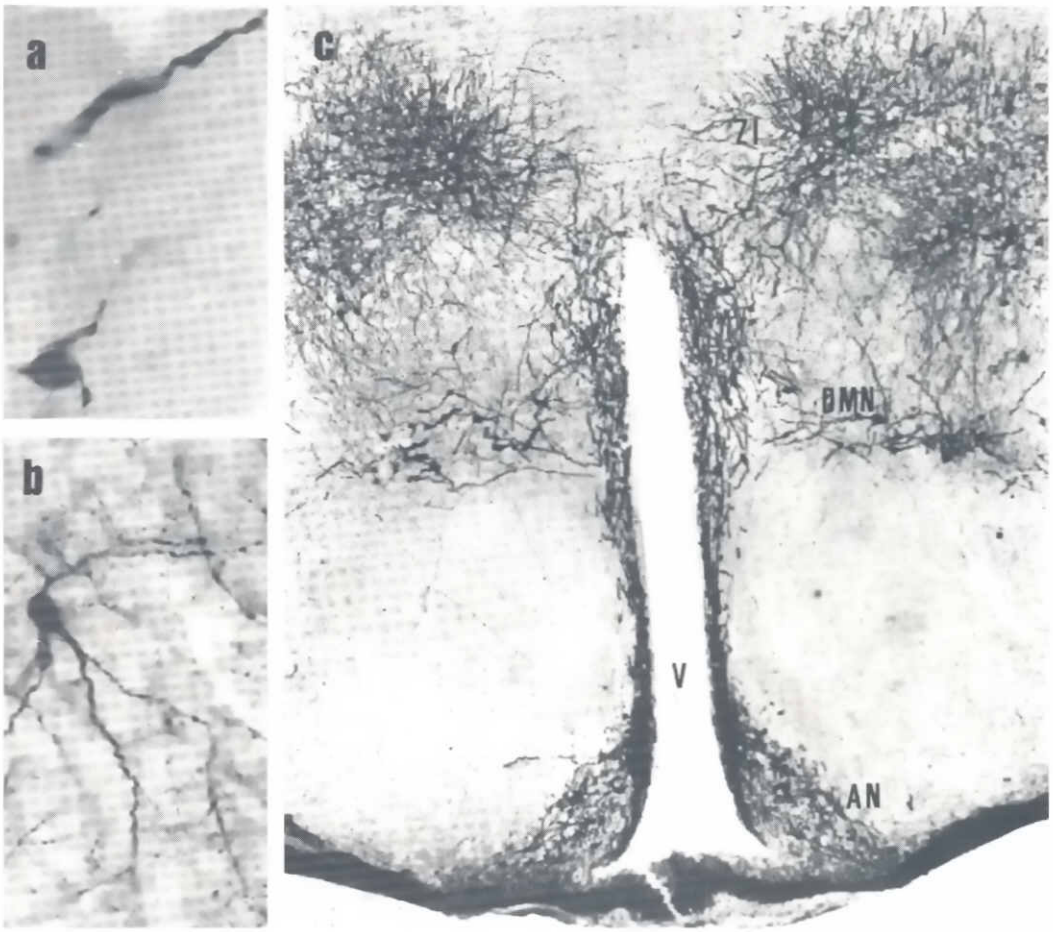


**Fig. 2. Lowly (a) and highly (b,c) differentiated tyrosine hydroxylase-immunoreactive neurons in fetal (a) and young (b,c) rats.** *AN, arcuate nucleus; DMN, dorsomedial nucleus; ZI, zona incerta; V, third ventricle.*

According to our suggestion, 5-HT-IR neurons could serve as a temporal store of 5-HT either released from the 5-HT axons of the medial forebrain bundle, or circulated in the cerebrospinal fluid (Ugrumov *et al.*, 1985, 1989b). As to 5-HT-IR cells in the anterolateral hypothalamus of perinatal rats, they most probably represent the transient neuronal population, partly expressed phenotype of 5-HT neurons (Ugrumov *et al.*, 1989b). On the other hand, such characteristics as an ability for specific uptake and decarboxylation of the 5-HT precursor make these neurons similar to APUD cells (Pearse, 1986).

***Monoaminergic fibers.***

5-HT and CArgic fibers descending from the mesencephalon and brainstem first reach the hypothalamus in rats at E13-E14. By the end of fetal life, MArgic fibers arrive in the target neurosecretory nuclei, the median eminence and the intermediate lobe giving rise to axo-neuronal, axo-vascular, axo-axonal, axo-ventricular and axo-glandular contacts (Ugrumov, 1991, 1992). The density of the hypothalamic fiber network increases progressively during the perinatal period coinciding with the establishment of specialized contacts with target neurons, first, of presynapses (immature synapses) and, then, of symmetric and asymmetric synapses (Ugrumov *et al.*, 1986, 1989a,d, 1994a,b; Borisova *et al.*, 1991). It is noteworthy that the suprachiasmatic nucleus of young rats (P2-P10) receives an intense innervation either by provisional TH-IR fibers, or by the fibers with transient expression of TH (Ugrumov *et al.*, 1989d; Beltramo *et al.*, 1994).

Thus, DArgic neurons first appear in the hypothalamus long before birth occupying their definitive positions in the zona incerta, periventricular and arcuate nuclei over the perinatal period. Moreover, the developing hypothalamus contains the neurons which partly express the 5-HT phenotype. From E12 until puberty, the hypothalamus becomes innervated by CArgic and 5-HT fibers, belonging either to hypothalamic neurons or arriving via the medial forebrain bundle from the brainstem and mesencephalon. The fibers innervate the target neurosecretory nuclei and sprout to the median eminence, third ventricle and intermediate lobe.

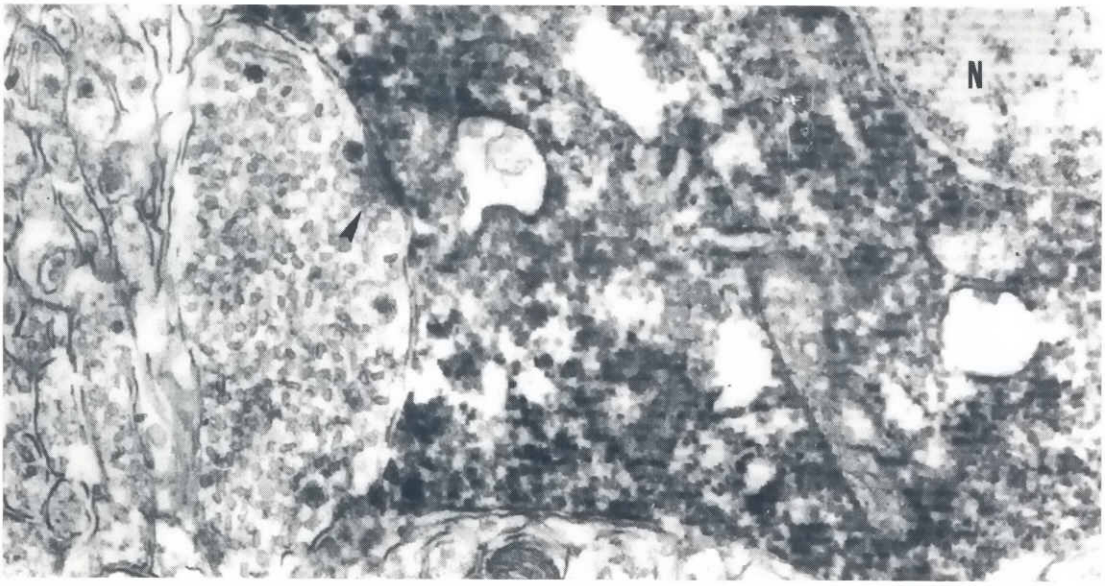


**Fig. 3. Axo-somatic asymmetric synapse (*arrowhead*) between tyrosine hydroxylase-immunoreactive cell body and the immunonegative axon.** *N, nucleus.*

### Pharmacological perturbations of the monoamine metabolism

#### *Inhibition of the catecholamine system*

Although MAs are considered as potent morphogens (Lauder, 1993), their role in the development of the target brain regions, still, remains far from complete understanding because of technical difficulties. Indeed, the approaches used to evaluate the MA functional significance in adult mammals, such as stereotactic injections of drugs, interfering with the MA metabolism into the local brain regions or cerebral ventricles, microsurgical brain lesions, etc. are not applicable to fetuses and early postnatal animals (Jonsson, 1983). Therefore, we have used pharmacological models of MA depletion in the hypothalamus of fetuses or young rats by systemic (subcutaneous or intraperitoneal) injections of the inhibitors of the MA synthesis and neurotoxins (Bernabe *et al.*, 1996).

The treatment of pregnant rats and, hence, fetuses with α-methyl-m(p)-tyrosine, an inhibitor of CA synthesis, from E12 to E20 caused a fall for 50% in the hypothalamic noradrenaline and adrenaline concentrations while the DA level remained unchanged (Bernabe *et al.*, 1996). 6-Hydroxydopamine, a neurotoxin, could not be applied to fetal materials as it does not penetrate through the maternal-fetal placental barrier (Jonsson, 1983).

Daily injections of α-methyl-m(p)-tyrosine to rats from P2 to P10 resulted in a 4-fold decreased noradrenaline level and 2-fold decreased adrenaline level, while the DA concentration remained unchanged. Still, additional daily injections of 6-hydroxydopamine depleted hypothalamic DA by approximately 25% (Bernabe *et al.*, 1996). The efficacy of the neurotoxin treatment of neonatal rats is partly explained by a high permeability of the blood-brain barrier at least until P10 (Jonsson, 1983).

#### *Inhibition of the serotoninergic system*

The 5-HT system of fetuses and young rats was suppressed by p-chlorophenylalanine, an inhibitor of 5-HT synthesis. The efficacy of this pharmacological model has been proved earlier showing a significant inhibition of tryptophan hydroxylase and depletion of 5-HT in the fetal brain (for ref. Lauder, 1993). In our studies, the fetuses were treated with this inhibitor from E8-E12 until E20 and young rats from P2 to P10 (Ugrumov *et al.*, 1986).

Thus, pharmacological models used in this study occurred to be valuable for inhibition of MArgic systems in the developing hypothalamus of fetuses and young rats.

### Functional significance of hypothalamic catecholamines

#### *Autoregulation*

The development of the hypothalamic CArgic system in the animals treated with α-methyl-m(p)-tyrosine has been specified by evaluating CA specific uptake (Bernabe *et al.*, 1996). The specific [$^3$H]MA uptake by the nervous tissue is considered as an index of the MArgic innervation, i.e. the value of [$^3$H]MA uptake is proportional to the total axonal membrane areas and, hence, to the number and size of axonal terminals (Sachs and Jonsson, 1975; Borisova *et al.*, 1991).

According to our data, the [$^3$H]DA uptake by the hypothalamic tissue at E21 decreased 50% following CA depletion, suggesting a CA stimulatory effect on the developing CArgic system, e.g. on the axonal growing and ramifications (Bernabe *et al.*, 1996). The effect of the CA depletion on the developing CArgic system appears to be specific since the CA deficiency does not influence the DA uptake by the hypothalamic tissue of young rats while provides a stimulatory effect on the peptidergic target neurons (see below).

#### *Catecholamine influence on the peptidergic target neurons*

The vasopressinergic and oxytocinergic neurons of the supraoptic nucleus, highly innervated by CArgic axons in adults, were chosen to evaluate the CA influence on their differentiation. According to *in situ* hybridization, the CA depletion does not modify the concentrations of vasopressin and oxytocin mRNAs in fetuses while resulted in a significant augmentation of both mRNAs in

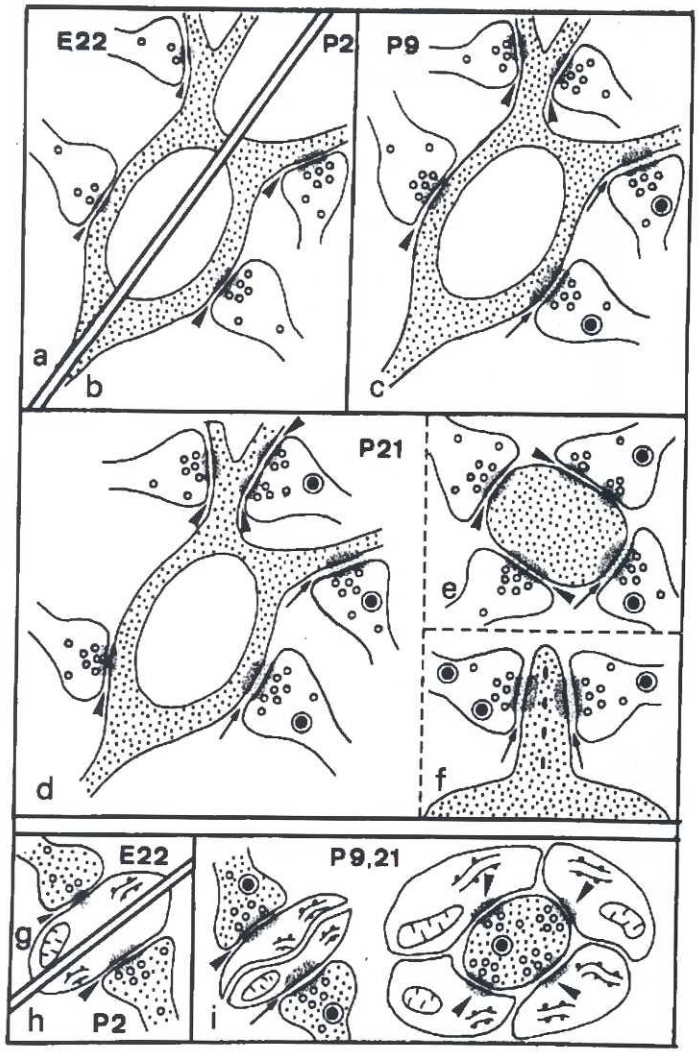


**Fig. 4. Schematic representation of synaptogenesis in the hypothalamus of fetal (E22: a,g) and postnatal (P2: b,h; P9: c,i; P21: d-f,i) rats.** *Presynapses and synapses are made either by tyrosine hydroxylase-immunoreactive cell bodies and fibers (dots) with immunonegative axons (**a-f**) or by tyrosine hydroxylase-immunoreactive axons (dots) and immunonegative dendrites (**g-i**). Small arrowhead, presynapse; large arrowhead, symmetric synapse; arrow, asymmetric synapse. (Adapted from Ugrumov et al., 1994a).*

young rats (Fig. 6) (Ugrumov *et al.*, 1994c; Trembleau *et al.*, 1995). These data suggest the CA inhibitory influence on the peptide gene expression, but only during the early postnatal period corresponding to synaptogenesis (Ugrumov, 1994). Therefore, it is tempting to suppose that CAs provide their action via synapses. It should be stressed that the augmentation of the vasopressin mRNA level in treated young rats twice exceeds that of oxytocin mRNA level. This difference, apparently, is a result of a more intense CArgic innervation of vasopressinergic neurons compared to that of oxytocinergic neurons (Sladek and Armstrong, 1987) and, hence, of a more acute CA control of vasopressin gene expression. According to our preliminary data, the CA depletion during the perinatal period provides an imprinting (irreversible) effect, at least, on the differentiating vasopressinergic neurons (Beltramo *et al.*, accepted).

## Functional significance of hypothalamic serotonin

### *Autoregulation*

The development of the hypothalamic 5-HT system has been specified according to [$^3$H]5-HT uptake and release *in vitro* in fetal and young rats following 5-HT depletion by p-chlorophenylalanine. The 5-HT specific uptake was not changed in fetuses and young rats in response to chronic treatment with p-chlorophenylalanine. On the contrary, the spontaneous and $K^+$-induced $CA^{2+}$-dependent release of [$^3$H]5-HT decreased in 2.5 times in fetuses, but not in neonates. From these data follows that 5-HT, as CAs (see above), stimulates the development of the 5-HT system in fetuses, but the mechanisms of the actions of 5-HT and CAs are different. (Sapronova *et al.*, unpublished).

### *Serotonin influence on the peptidergic target neurons*

Vasoactive intestinal polypeptide neurons of the suprachiasmatic nucleus of adults receive strong innervation by the 5-HT axons, thus, being a good model for studying the 5-HT influence on the differentiating target neurons. The 5-HT depletion in rat fetuses

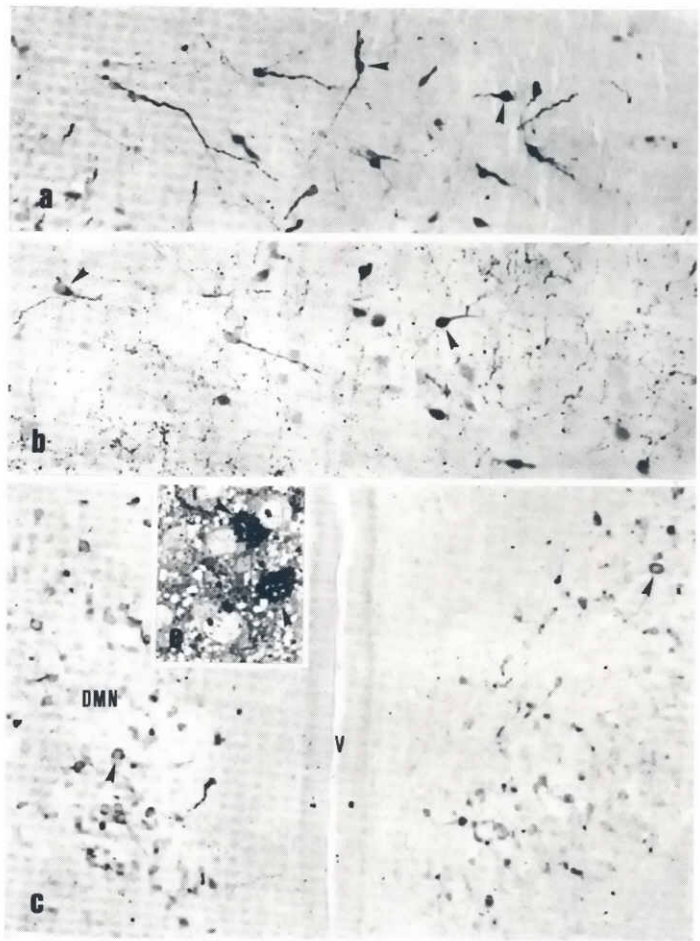


**Fig. 5. Serotonin-immunoreactive (a-c) and radioactively labeled (d) neurons (*arrowhead*) in the anterolateral hypothalamus (a, b) and dorsomedial nucleus (d) of fetal (a) and young (b-d) rats.** *DMN, dorsomedial nucleus; V, third ventricle.*

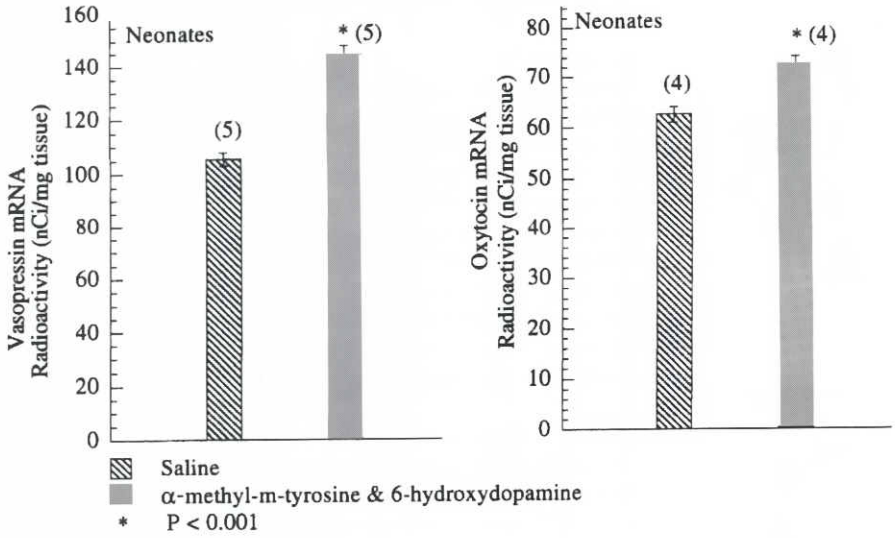


**Fig. 6. Contents of vasopressin and oxytocin mRNAs (radioactivity) in the supraoptic nucleus of young rats following chronic catecholamine depletion.**

during the second half of the intrauterine development resulted in a significant augmentation of the vasoactive intestinal polypeptide mRNA concentration (Fig. 7) while the same treatment of young rats from P2 to P10 was ineffective (Ugrumov *et al.*, 1994d). This suggests that 5-HT inhibits vasoactive intestinal polypeptide gene expression, but only in fetuses before synaptogenesis. It means that in contrast to the CA action on the vasopressin and oxytocin gene expressions, the 5-HT influence on the vasoactive intestinal polypeptide gene expression would not be transmitted via synaptic input (Ugrumov *et al.*, 1994c,d). Still, it remains to be elucidated whether 5-HT provides a programming or activating influence on target neurons.

Thus, MAs inhibit the peptide gene expression in the hypothalamic target neurons during critical periods of ontogenesis. Although final effects of different MAs are similar, the mechanisms of their actions appear to be different.

### *Serotonin influence on the developing gonadotropin-releasing hormone system*

The gonadotropin-releasing hormone neurons located in the septo-preoptic area are innervated by 5-HT axons, thus, being the targets for 5-HT (Ugrumov, 1992). Our recent study has evaluated the hypothalamo-hypophysial-gonadotropic activity in rats at E21 following the 5-HT depletion from E8 to E20 with p-chlorophenylalanine. Under 5-HT deficiency, the hypothalamic gonadotropin-releasing hormone level highly decreased in fetal males, but not in females (Fig. 7) (Adamskaya *et al.*, 1994). Simultaneous small but statistically significant increase of the plasma luteinizing hormone level in males, but not in females, suggests that: a) a fall of the hypothalamic gonadotropin-releasing hormone level is rather due to gonadotropin-releasing hormone release than to the inhibition of its synthesis; and b) 5-HT inhibiting control over gonadotropic function is established at least at E21, but only in males. Moreover, we cannot exclude that 5-HT controls migration of gonadotropin-releasing hormone neurons from the place of their origin in the nasal cranium to the septo-preoptic area.

We failed to explain the mechanisms of sex differences in gonadotropin-releasing hormone and luteinizing hormone responses to the 5-HT depletion. Apparently, they are not related to the activating (reversible) effects of sex steroids. In fact, chronic treatment of fetuses with p-chlorophenylalanine did not change testosterone levels both in plasma and testes (Adamskaya *et al.*, 1994). Nevertheless, these differences might be related either to the prenatal masculinizing action (irreversible) of androgens on the brain or to genetic sexual dimorphism in differentiating neurons.

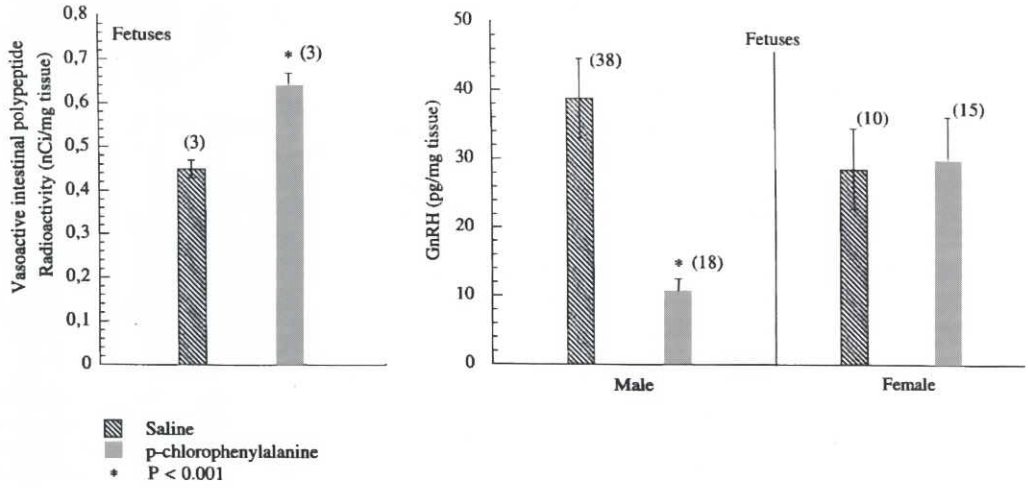


**Fig. 7. Contents of vasoactive intestinal polypeptide mRNA in the suprachiasmatic nucleus and gonadotropin-releasing hormone (GnRH) in the hypothalamus of fetal rats following chronic serotonin depletion.**

## Neurohumoral regulation of the development of the monoaminergic systems

### *Sex steroids*

In addition to MAs (see above), the hormones of the peripheral endocrine glands are involved in the regulation of the development of MArgic systems. Our study attempted to find sex differences in the adult 5-HT system as well as to evaluate the role of androgens in their appearance during ontogenesis (Borisova *et al.*, 1996). To this aim, the 5-HT content and specific uptake were measured in the hypothalamus in intact adult females and males as well as in neonatally castrated adult males. The 5-HT content and specific uptake in intact females exceed significantly those in intact males. Neonatal castration of males resulted in the increase of the 5-HT content and uptake to their levels in intact females. These data prove an inhibitory influence of androgens on the development of the hypothalamic 5-HT system that is apparently related to the neonatal masculinization of the brain (Borisova *et al.*, 1996).

### *Maternal and placental neurohumoral factors*

Besides of the fetal neurohumoral factors, prenatally the developing organism is under control by the maternal and placental neurohumoral factors. In order to evaluate the possible role of these factors in differentiation of DArgic neurons, the mediobasal hypothalamus of fetuses was grafted into the third ventricle of adult rats. Two and six months after operation, TH- and AADC-IR neurons were detected in the graft showing no visible differences in their number, size and distribution compared to the adult intact rats and recipients. The major population is represented by the small uni- and bipolar cells expressing TH, AADC or both enzymes. However, only the last population can be considered as the true DArgic neurons. The axons of the grafted neurons terminated either within the graft or in the recipient hypothalamus, preferentially in the regions being rich in myelinic fibers (optic chiasma, commissure) (Fetisov *et al.*, 1994; Ugrumov *et al.*, 1994e).

Thus, the phenotype expression of DArgic neurons at least of the arcuate nucleus is highly predetermined by the genetic program, and its realization does not depend significantly on the maternal and placental microenvironment.

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## References

ADAMSKAYA, E., SAPRONOVA, A., BABICHEV, V., PROSHLYAKOVA, E. and UGRUMOV, M. (1994). Effect of serotonin depletion on the hypothalamo-hypophysial-gonadotropic activity in rat fetuses. *Neuroendocrinol. Lett. 16*: 201-207.

BALAN, I.S., UGRUMOV, M.V., BORISOVA, N.A., CALAS, A, PILGRIM, C., REISERT, I. and THIBAULT, J. (1997). Birthdates of the tyrosine hydroxylase immunoreactive neurons in the rat hypothalamus with reference to sexual dimorphism. *Neuroendocrinology*, (in press).

BAUM, M.J., WOUTERSON, P.J.A. and SLOB, A.K. (1991). Sex difference in whole-body androgen content in rats on fetal days 18 and 19 without evidence that androgen passes from males to females. *Biol. Reprod. 44*: 747-751.

BELTRAMO, M., CALAS, A., CHERNIGOVSKAYA, N., BORISOVA, N., POLENOVA, O., TILLET, Y., THIBAULT, J. and UGRUMOV, M. (1994). Postnatal development of the suprachiasmatic nucleus in the rat. Morpho-functional characteristics and time course of tyrosine hydroxylase immunopositive fibers. *Neuroscience 63*: 603-610.

BELTRAMO, M., CALAS, A., CHERNIGOVSKAYA, E., ALONSO, J., THIBAULT, J. and UGRUMOV, M.V. (1997). Long-lasting effect of catecholamine deficiency on the differentiating vasopressin neurons in the rat supraoptic nucleus. *Neuroscience*, (accepted).

BERNABE, J., PROSHLYAKOVA, E., SAPRONOVA, A., TREMBLEAU, A., CALAS, A. and UGRUMOV, M. (1996). Pharmacological model of catecholamine depletion in the hypothalamus of fetal and neonatal rats and its application. *Cell. Mol. Neurobiol. 16*: 617-624.

BJÖRKLUND, A. and LINDVALL, O. (1984). Dopamine-containing systems in the CNS. In *Handbook of Chemical Neuroanatomy*. Vol. 2. *Classical Neurotransmitters in the CNS*. Part 1 (Eds. A. Björklund and T. Hökfelt). Elsevier, Amsterdam, pp. 55-122.

BORISOVA, N.A., SAPRONOVA, A.Y., PROSHLYAKOVA, E.V. and UGRUMOV, M.V. (1991). Ontogenesis of the hypothalamic catecholaminergic system in rats. Synthesis, uptake and release of catecholamines. *Neuroscience 43*: 223-229.

BORISOVA, N.A., UGRUMOV, M.V., BALAN, I.S. and THIBAULT, J. (1993). Development of the tuberoinfundibular system in rats: birthdates of the tyrosine hydroxylase-immunopositive neurons. *Dev. Brain Res. 73*: 173-176.

BORISOVA, N.A., PROSHLYAKOVA, E.V., SAPRONOVA, A.Ya. and UGRUMOV, M.V. (1996) Androgen dependent sex differences in the hypothalamic serotoninergic system. *Eur. J. Endocrinol. 134*: 232-235.

EVERITT, B.J., MEISTER, B. and HÖKFELT, T. (1992). The organization of monoaminergic neurons in the hypothalamus in relation to neuroendocrine integration. In *Neuroendocrinology* (Ed. C.B. Nemeroff). CRS Press, Boca Raton, pp. 87-128.

FALCK, B., HILLARP, N.A., THIEME, G. and TORP, A. (1962). Fluorescence of catecholamines and related compounds condensed with formaldehyde. *J. Histochem. Cytochem. 10*: 348-354.

FETISOV, S.O., POPOV, A.P., EFUNI, E.S., THIBAULT, J., KRIEGER, M. and UGRUMOV, M.V. (1994). Expression of the dopaminergic phenotype by the neurons of the hypothalamic arcuate nucleus in the graft. *Proc. Russ. Acad. Sci (Dokl. Russ. Akad. Nauk), 334:* 124-126.

JONSSON, G. (1983). Chemical lesioning techniques: monoamine neurotoxin. In *Handbook of Neuroanatomy*. Vol. 1. *Methods in Chemical Neuroanatomy* (Eds. A. Björklund and T. Hökfelt). Elsevier, Amsterdam, pp. 463-507.

LAUDER, J. M. (1993). Neurotransmitters as growth regulatory signals: role of receptors and second messengers. *Trends Neurosci. 16*: 233-240.

LAUDER, J.M. and BLOOM, F.E. (1974). Ontogeny of monoamine neurons in the locus coeruleus, raphe nuclei, and substantia nigra of the rat. I. Cell differentiation. *J. Comp. Neurol. 155*: 469-481.

PEARSE, A.G.E. (1986). The diffuse neuroendocrine system: peptides, amines, placodes and the APUD theory. *Prog. Brain Res. 68*: 25-31.

SACHS, C. and JONSSON, G. (1975). Effects of 6-hydroxydopamine on central noradrenaline neurons during ontogeny. *Brain Res. 99*: 277-291.

SLADEK, C.D. and ARMSTRONG, W.E. (1987). Effect of neurotransmitters and neuropeptides on vasopressin release. In *Vasopressin; Principles & Properties* (Eds. D.M. Gash and G.J. Boer). Plenum Publ., New York, pp. 275-333.

STEINBUSCH, H.W.M. and NIEUWENHUYS, R. (1981). Localization of serotonin-like immunoreactivity in the central nervous system and pituitary of the rat, with special reference to the innervation of the hypothalamus. In *Serotonin: Current Aspects of Neurochemistry and Function* (Eds. B. Haber, S. Gabay, M.R. Issidorides and S.G.A. Alivisatos). Plenum, New York, pp. 7-35.

TREMBLEAU, A., UGRUMOV, M., ROCHE, D. and CALAS, A. (1995). Vasopressin and oxytocin gene expressions in intact rats and under the catecholamine deficiency during ontogenesis. *Brain Res. Bull. 37*: 437-448.

UGRUMOV, M.V. (1991). Developing hypothalamus in differentiation of neurosecretory neurons and in establishment of pathways for neurohormone transport. *Int. Rev. Cytol. 129*: 207-267.

UGRUMOV, M.V. (1992). Development of the hypothalamic monoaminergic system in ontogenesis. Morpho-functional aspects. *Zool. Sci. 9*: 17-36.

UGRUMOV, M.V. (1994). Hypothalamic catecholaminergic systems in ontogenesis: development and functional significance. In *Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates* (Eds. W.J.A.J. Smeets and A. Reiner). Cambridge Univ. Press, Cambridge, pp. 435-452.

UGRUMOV, M.V., TAXI, J., MITSKEVICH, M.S., ARLUISON, M., and TRAMU, G. (1985). Immunocytochemical and radioautographic study of serotonin projections to cerebral ventricles of perinatal rats. *Dev. Brain Res. 18*: 225-230.

UGRUMOV, M.V., TAXI, J., MITSKEVICH, M.S. and TRAMU, G. (1986). Development of the hypothalamic serotoninergic system during ontogenesis in rats. Immunocytochemical and radioautographic study. *Dev. Brain Res. 30*: 75-84.

UGRUMOV, M.V., TAXI, J., STEINBUSCH, H.W.M., TRAMU, G. and MITSKEVICH, M.S. (1988). Sur la signification de la réaction positive à l'anti-serotonine (5-HT) de certains neurons hypothalamiques du rat dans la période perinatale. *C.R. Acad. Sci. Paris 303*: 505-509.

UGRUMOV, M.V., PROSHLYAKOVA, E.V. and SAPRONOVA, A.Y. (1989a). Development of the hypothalamic 5-HT system during ontogenesis in rats: uptake and release of 5-HT in vitro. *Neuroscience 32*: 127-131.

UGRUMOV, M.V., TAXI, J., STEINBUSCH, H.W.M., TRAMU, G. and MITSKEVICH, M.S. (1989b). On the distribution and morpho-functional characteristics of 5-HT-immunoreactive cells in the hypothalamus of fetuses and neonatal rats. *Dev. Brain Res. 46*: 233-241.

UGRUMOV, M.V., TAXI, J., TIXIER-VIDAL, A., THIBAULT, J. and MITSKEVICH, M.S. (1989c). Ontogenesis of tyrosine hydroxylase-immunopositive structures in the rat hypothalamus. An atlas of neuronal cell bodies. *Neuroscience 29*: 135-156.

UGRUMOV, M.V., TIXIER-VIDAL, A., TAXI, J., THIBAULT, J. and MITSKEVICH, M.S. (1989d). Ontogenesis of tyrosine hydroxylase-immunopositive structures in the rat hypothalamus. Fiber pathways and terminal fields. *Neuroscience 29*: 157-166.

UGRUMOV, M.V., POPOV, A.P., VLADIMIROV, S.V., KASMAMBETOVA, S. and THIBAULT, J. (1994a). Development of the suprachiasmatic nucleus during ontogenesis. Tyrosine hydroxylase immunopositive cell bodies and fibers. *Neuroscience 58*: 151-160.

UGRUMOV, M.V., POPOV, A.P., VLADIMIROV, S.V., KASMAMBETOVA, S., NOVODJILOVA, A.P. and TRAMU, G. (1994b). Development of the suprachiasmatic nucleus during ontogenesis. Serotonin immunopositive fibers. *Neuroscience 58*: 161-165.

UGRUMOV, M.V., TREMBLEAU, A., ROCHE, D. and CALAS, A. (1994c). Monoamine influence on neuropeptide gene expression during ontogenesis. *Acta Biol. Hung. 45*: 441-450.

UGRUMOV, M.V., TREMBLEAU, A. and CALAS A. (1994d). Altered vasoactive intestinal polypeptide gene expression in the fetal rat suprachiasmatic nucleus following prenatal serotonin deficiency. *Int. J. Dev. Neurosci.12*: 143-149.

UGRUMOV, M.V., FETISOV, S.O., POPOV, A.P., EFUNI, E.S., TITOVA, N.G., THIBAULT J. and KRIEGER M. (1994e). Development of the embryonic mediobasal hypothalamus grafted into the 3rd ventricle of the adult rat. News Russ. Acad. Sci (Investya Ross. Akad. Nauk), *Ser. Biol. 3*: 340-351.

WALLACE, J.A. and LAUDER, J.M. (1992). Development of the serotoninergic system in rat and chick embryos. In *Handbook of Chemical Neuroanatomy*.Vol. 10: *Ontogeny of Transmitters and Peptides in the CNS* (Eds. A. Björklund, T. Hökfelt and M. Tohyama). Elsevier, Amsterdam, pp. 619-645.

WEINER, R.I., FINDELL, P.R. and KORDON, C. (1988). Role of classic and peptide neuromediators in the neuroendocrine regulation of LH and prolactin. In *The Physiology of Reproduction* (Eds. E. Knobil, J. Neill, et al.). Raven Press, New York, pp. 1235-1281.