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# Development of Male Sexual Function After Prenatal Modulation of Cholinergic System

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## 1. Introduction

Embryonal period of ontogenesis plays an important role in the brain development which is defined, first of all, by genetical factors. Normal flow of the process can be disturbed also under the influence of many environmental factors which affect, both a differentiation of neurones, and on a neurotransmitter choice in them used for communications with the proximate cells (Le Douarin, 1981; Pendleton, 1998). The majority of the factors attacking developing brain during this period, break a normal ontogenesis of neurotransmitter systems: NA, 5-HT, DA and ACh that shows high sensitivity of a brain in critical periods of the development (Williams, 1992; Oliff, 1999; Qiao, 2004).

A variety of neurochemical changes in the embryonic brain, induced by exposure to neurotropic compounds during the prenatal period, result in the development of functional impairments and behavioral disorders in the adult offspring. The mechanisms of action of many chemical factors on the developing fetal brain during early ontogenesis are in most cases mediated by alterations in the formation and functioning of brain neurotransmitter systems, including the cholinergic system, whose CNS function is associated with memory, learning, and behavioral processes (Yamada et al., 1986; Buzsaki, 1989; Everitt & Robbins, 1998; Levin & Slotkin, 1988; Zoli et al., 1999). During the period of neuron development, actions on cholinergic mechanisms lead to delays in cell differentiation which correlate with cognitive and behavioral deficits in fertile offspring (Yamada et al., 1986; Levin & Simon, 1998; Beer et al., 2005).

Prenatal exposure to neurotoxins (nicotine, organochlorine compounds, barbiturates), which have cholinotropic properties, produces long-lasting changes in neurotransmitter functions in early ontogenesis with the subsequent development of neurobehavioral anomalies and affective disorders in pubescent individuals (Seidler et al., 1992; Barinaga, 1996; Slotkin, 2004). Thus, embryonic exposure to nicotine leads to alterations to cell proliferation and differentiation, resulting in long-term changes to synaptic function (Peters, 1986; Lichtensteiger, 1988). Binding to N-cholinergic receptors in catecholamine-containing neurons in the fetal brain, nicotine disrupts the expression of these transmitters

(Lichtensteiger et al., 1988; Dani & Heinemann, 1996). Prenatal exposure to nicotine predominantly damages cholinergic, noradrenergic, and dopaminergic projections in the brain during postnatal life, with later cognitive and behavioral dysfunction in adult offspring (Naeye & Peters, 1984; Milberger et al., 1996; Fergusson et al., 1998; Orlebeke et al., 1997; Weissman et al., 1999; Slotkin et al., 2001).

The neurobehavioral teratogenic actions of barbiturates are also mediated by disrupted functioning of septohippocampal cholinergic conducting pathways, which is accompanied by a deficiency of synaptic transmission and accompanying hippocampus-related behavioral deficit (Wallace, 1984; Smith et al., 1986; Yanai, 1984, 1996; Steingart et al., 2000; Azmitia, 2001; Beer et al., 2005; Beer et al., 2005). Phenobarbital, previously used for the prophylaxis of neonatal hyperbilirubinemia and bleeding in neonatal children, is a teratogenic factor in relation to behavior in both humans and animals (Yanai, 1984; Wallace, 1984; Smith et al., 1986). Experimental exposure to organochlorine compounds, like other neurotoxins with cholinotropic properties, damages cholinergic conducting pathways and leads to long-term alterations to the cholinergic system (Lauder, 1985; Dreyfus, 1998; Qiao et al., 2002; Slotkin et al., 2002; Slotkin, 2004). Dysfunction of cholinergic neurons plays a significant role in behavioral disorders seen in adult rats given organochlorine compounds during the prenatal period (Sherman et al., 1981).

Behavioral abnormalities as long-term consequences of prenatal exposure to various factors are generally difficult to observe because of the large phenotypic variability of the developing organism (Nicholls, 2000). There is great value in studying sexual behavior in these situations, as sexual functions are the most sensitive and susceptible aspects of reproduction in males and, as has been demonstrated, are regulated by the activities of several neurotransmitter systems, including the cholinergic (Bitran & Hull, 1987; Mas et al., 1987; Hull et al., 1988; Retana et al., 1993; Gladkova, 2000).

In addition, despite many studies of substances with cholinotropic properties and adverse influences on the developing brain during the prenatal period, the literature lacks reports of the behavioral effects of prenatally administered selective cholinolytics.

Taking into consideration all these statements, the purpose of the present research is study of known selective blockers of M- and N-cholinergic systems, prescribed in various terms of a prenatal period, on development dynamics of neurotransmitter systems of the rats embryos brain, and track their dynamics in an adolescent period and in adulthood in comparison to the behavioural sexual status in the past for a sexual behavior at rats males.

Tasks of the present part of work were the following:

- Research of sexual abnormalities of the 3-4-month-old rats males and possibility of their pharmacological correction.
- Analysis of the effects of selective M- and N-cholinergic systems blockers prenatal influence on dynamics of neurotransmitter systems development of 20-day-old rats embryos brain, in various terms of a gestation.
- Study of the neurochemical status of brain structures, participating in formation and regulation of neuroendocrinal and behavioural function of 2-month-old rats that were effected by prenatal influence M- and N-cholinolytics.

## 2. Methods

Investigations were performed on Wistar rats from the Rappolovo supplier, Russian Academy of Medical Sciences (Leningradskaya Oblast). Several series of experiments were

performed. Female rats with a known date of pregnancy were obtained by mating females in proestrus-estrus with males. The day on which sperm were seen in vaginal smears was taken as the first day of pregnancy. Pregnant females, at different stages of gestation (9–11, 12–14, and 17–19 days of pregnancy), were given three i.m. injections (once daily) of the N-cholinoblocker ganglerone (10 mg/kg), while other groups received injections of the M-cholinoblocker methylbenactyzine (2 mg/kg) at the same time points. Doses were determined on the basis of the selectivities of cholinolytic actions and the absence of nonspecific actions. Control groups of females received injections of physiological saline. Experiments offspring groups (12–14 individuals per group) were formed in accord with the timing of prenatal administration of ganglerone (groups G10, G13, and G18, respectively) and methylbenactyzine (groups M10, M13, and M18). The offspring of intact rats served as the control group.

**Behavioral studies** were performed on rat offspring aged 3.5–4 months. Sexual experience was acquired in four sequential tests with receptive females. Sexual activity parameters were assessed using a standard sexual behavior test (3). Adult rats were kept in individual cages with food and water available *ad libitum*, in a room with controlled temperature and under an inverted 12 × 12 h light cycle (light off at 09:00 h). Tests for sexual behavior were done during the dark phase of the cycle and under dim red light illumination. The test male was placed in the study chamber, of size 40 × 40 × 30 cm, for 5 min prior to presentation of a sexually susceptible female. Experiments were performed in dim red illumination. Receptivity in sterilized females was induced by sequential administration of estradiol dipropionate (25 mg, 48 h before the experiment) and progesterone (500 g, 4 h before the experiment). Components of sexual activity were recorded visually for 15 min in tests 1 and 4. The numbers of components of sexual behavior (mountings, intromissions, and ejaculations) and their latent periods were registered. During each behavioral test, the behavioral components recorded were mount latency (time from the introduction of a receptive female to the first mount), intromission latency (time from the introduction of a receptive female to the first intromission), ejaculation latency (interval between the first intromission and ejaculation), and postejaculatory interval (interval between the first ejaculation and the next intromission).

**Neurochemical studies** were performed using brains from 20-day embryos and brain structures (hypothalamus, hippocampus) from rat offspring two month age. The concentrations of the neurotransmitters dopamine (DA), noradrenaline (NA), and serotonin (5-HT) in brain tissues were measured by high-performance liquid chromatography using a Beckman System Gold with an LC-4C electrochemical detector. Brain structures were extracted on a cryostat at -20°C and were stored in liquid nitrogen until chromatographic analysis. Peaks were separated on a SphereClone 5 μ ODS 2 chromatography column (250 × 4.60 mm) with a Phenomenex precolumn. The mobile phase consisted of citratephosphate buffer pH 3.5, acetonitrile (88 ml/liter), and octanesulfonic acid (43 mg/liter). Chromatographic peaks were identified and assessed quantitatively in relation to peaks obtained from internal standards.

Serum hormone levels were assayed by immunoenzyme analysis using standard biochemical kits (Chema, Access) on a Uniplan immunoenzyme analyzer. Statistical analysis. Results were compared with control data and analyzed statistically by analysis of variance (ANOVA) on Origin 7.0.

### 3. Sexual function of 3,5-4-month-old males offsprings subjected to prenatal exposure of selective M- and N-cholinoblockers

For the purpose of revealing sexual function abnormalities of puberal offsprings of male rats their primary sexual activity and dynamics of acquisition of sexual experience has been investigated. Offsprings of intact rats were control group.

The results obtained from behavioral studies showed that administration of methylbenactyzine and ganglerone to pregnant females at different periods of gestation induced long-term impairments to sexual function in pubescent offspring. In the first test, there were significant reductions in sexual function on appearance of primary sexual activity in offspring subjected to prenatal exposure to ganglerone (groups G10–G18) (Fig. 1). The males of this group included the large proportion of individuals in which all elements of sexual behavior were absent (henceforth – “inactive” individuals). The proportion of inactive males among the offspring of groups M10–M18 was significantly smaller and, after acquisition of sexual experience, decreased to 1–2 individuals in terms of both copulative (Fig. 1, B) and ejaculatory (Fig. 1, A) functions.

As not all males demonstrated sexual activity including the final component of sexual behavior, i.e., ejaculation, a group of males showing incomplete copulatory activity (mounting and intromission, without ejaculation) was identified (Fig. 1, B).

Analysis of the elements of sexual behavior over time (from test 1 to test 4) showed that the most marked differences in the intensity of acquiring sexual experience between offspring in groups G10–G18 and M10–M18 were seen in relation to the final element of copulation, i.e., ejaculatory activity. Offspring of groups G10–G18 showed positive dynamics only for copulatory activity, while there were no changes in the amounts of ejaculatory activity with the increase in sexual experience. The proportions of inactive males in terms of this parameters in groups G10–G18 amounted to about half of the total number of animals (from four to seven individuals) used for testing sexual behavior (Fig. 1).

Changes in the time parameters of sexual functions during four sessions of sexual behavior showed a similar dynamic for copulatory components. As sexual experience was acquired, the latent periods of the main elements of sexual behavior decreased, to a greater extent in males of groups M10–M18 than in offspring of groups G10–G18, and were comparable with control values (for example, mounting latencies are shown in Fig. 2).

Thus, more males which were inactive in the sexual behavior test (both before and after acquisition of sexual experience) were seen among the offspring of groups G10–G18 (particularly in males of group G10). A characteristic feature of the sexual behavior of males in these groups was the absence of marked dynamics of the acquisition of sexual experience by the fourth test.

Studies of the structure of sexual behavior in males with acquired sexual experience showed that sexual function in males of groups G10–G18 was characterized by very low values for the copulatory components of sexual behavior, with long latent periods. Levels of ejaculatory activity were extremely low ( $0.40 \pm 0.16$  ejaculations among the G10 offspring compared with  $1.90 \pm 0.18$  in the control group).

The long latency of mounting in males of group G10 and, to a lesser extent, group G13, provides evidence of a significant alteration in the motivational component of sexual behavior (Fig. 2). In males of groups M10–M18 with acquired sexual experience, differences in sexual activity as compared with control offspring were less marked, with the exception of group M10, in which there was a significantly lower value of the ejaculatory component, without any change in the latent period of mounting. Analysis of the effects of prenatal

administration of substances at different periods of pregnancy showed that sexual function in the offspring was most sensitive to injection of the N-cholinolytic ganglerone at 9–11 and 12–14 days of gestation and the M-cholinolytic methylbenactyzine at 9–11 days of gestation. The comparative analysis of the other parameters of males sexual behaviour from the groups G10–G18 and M10–M18 with the acquired sexual experience showed more appreciable sexual dysfunctions of offsprings G10–G18 in comparison with the offsprings, that were subjected to prenatal influence of methylbenactyzine (tab. 1). The structure of sexual function of males from group G10–G18 with the acquired sexual experience was characterized by very low value of copulatory components and their high latence. Level of ejaculatory activity of all groups G10–G18 was extremely low and authentically differed from control group.

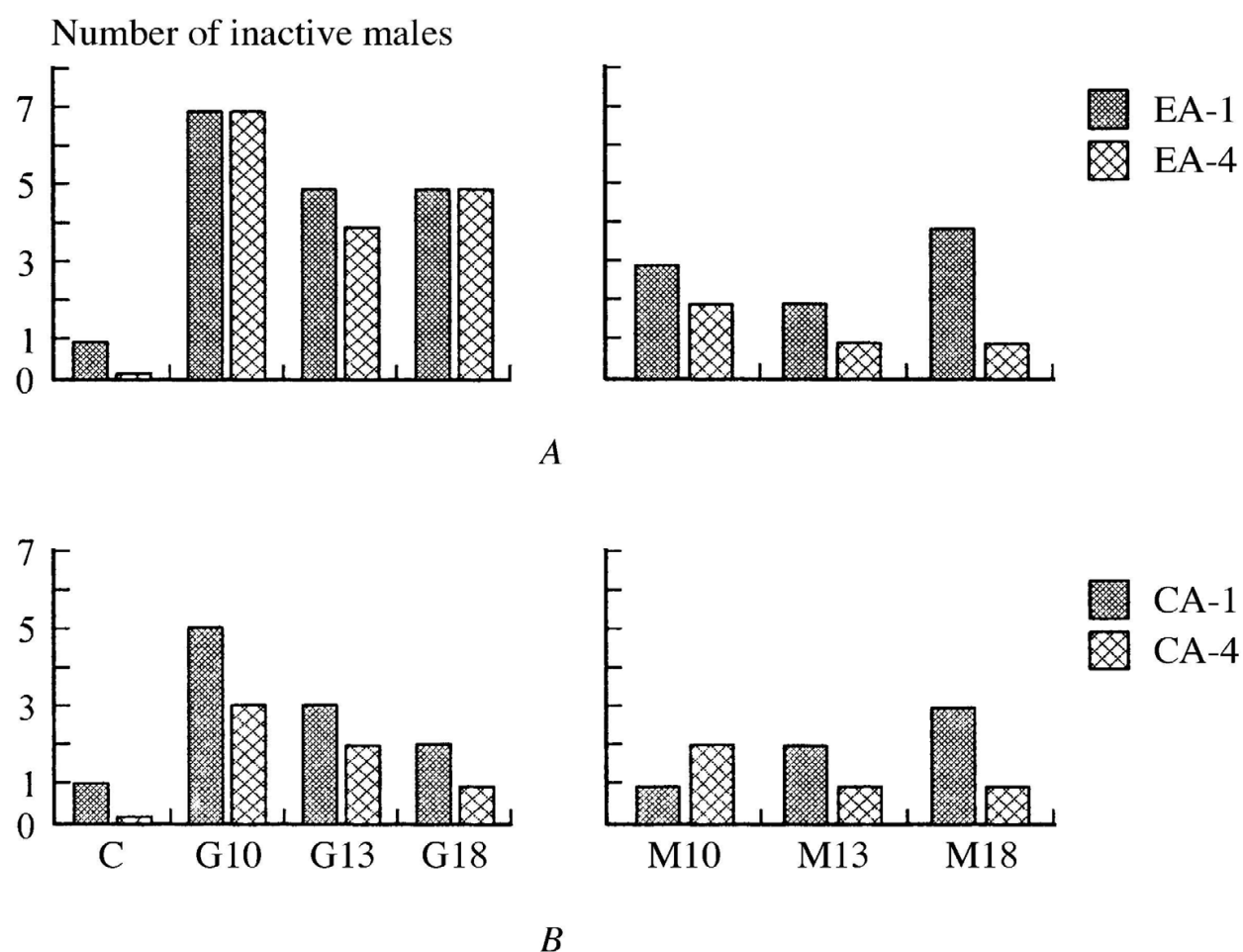


Fig. 1. Dynamics of the acquisition of sexual activity in offspring; data based on ejaculatory and copulatory (mountings and intromissions) behavior in four sequential tests for sexual behavior (data from tests 1 and 4) ( $n = 14$ ). Dark columns show numbers of inactive males in the first test; shaded columns show inactive males in the fourth test. **A**) Number of inactive males in terms of ejaculatory activity (EA) in offspring subjected to prenatal exposure to ganglerone at 9–11, 12–14, and 17–19 days of pregnancy (groups G10, G13, and G18, respectively) or methylbenactyzine (groups M10, M13, and M18, respectively) compared with control offspring; **B**) number of inactive males in terms of incomplete copulatory activity (CA) (mountings and intromissions) in offspring of these groups.

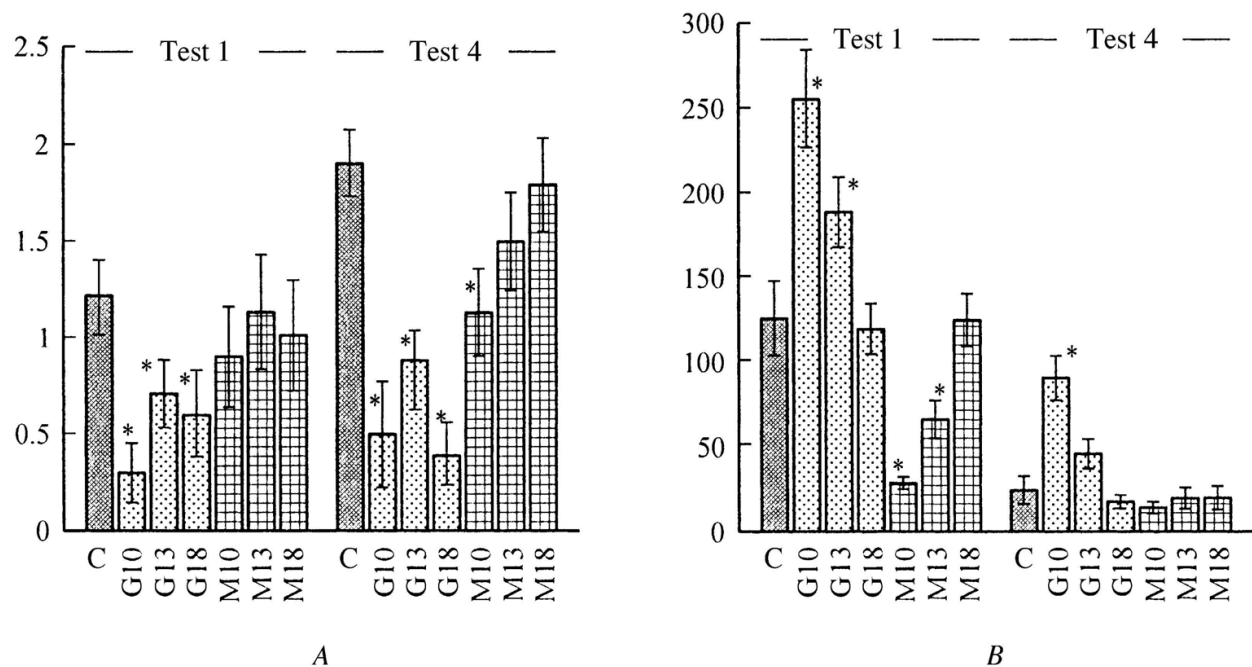


Fig. 2. Parameters of sexual behavior in the offspring of rats subjected to prenatal exposure to methylbenactyzine (M) or ganglerone (G) in the first and fourth tests. A) Latent period of mating. The ordinate shows time, sec; B) number of ejaculations throughout the sexual behavior test period. \* $p < 0.05$  compared with the control group. For further details see caption to Fig. 1.

Thus, the results of the present study show that treatment of pregnant females with ganglerone (and, to a lesser extent, methylbenactyzine) at different periods of pregnancy leads to behavioral abnormalities in the offspring: there were significant reductions in sexual function and the intensity of the acquisition of sexual experience.

The studies of Gladkova (1994) showed that the intensity of sexual behavior in males depends on having the appropriate experience. As a rule, sexual activity in the first test with receptive females was low, but increased from test to test such that the quantitative levels of sexuality in male rats were essentially constant after the third contact with females. Administration of the N-cholinolytic to pregnant females at 9–11 and 12–14 days of gestation facilitated the appearance of significantly larger proportions of inactive males among their offspring, these animals being characterized by an extremely low dynamic of the acquisition of sexual experience as compared with control offspring. In these males (groups G10–G13), acquisition of sexual experience was followed by sexual functions with significant disruption of ejaculatory activity and the central motivational element of sexual behavior. These data indicate that ganglerone modulation of the N-cholinergic system in the developing fetal brain leads to changes in the quantitative and qualitative characteristics of elements of sexual behavior in pubescent offspring.

In groups with prenatal exposure to the M-cholinolytic, changes in sexual function were noted only in offspring of group M10 – which had a significantly reduced level of the ejaculatory component as compared with control offspring, though this was not linked with changes in the motivational aspect of sexual behavior. These data showed that prenatal changes in the activity of the M-cholinergic system lead to insignificant behavioral consequences in pubescent offspring. Thus, the results obtained here provide evidence that

prenatal alterations to the N-cholinergic and, to a lesser extent, the M-cholinergic system induce long-term sexual impairments in the pubescent offspring.

Groups	Mounts (n)	latency of mount (sec)	Intrusions (n)	latency of intrusion (sec)	Ejaculation (n)	latency of ejaculation (sec)	Interejaculatory interval (sec)	Restoration period (sec)
Control_1	9,70 ±1,93	123,2±42,8	6,10±1,08	166,2±42,6	1,20±0,20	369,8±65,0	504,0±49,5	328,0±26,7
Control_4	15,8±2,15	23,6±28,3	9,50±1,65	73,8±35,8	1,90±0,18	297,4±48,9	362,6±39,5	270,3±15,6
G-10_1	6,8±2,70	255,6±149,3	3,90±1,80	320,8±139,3	0,30±0,15	449,0±44,2	-	288±39,7
G-10_4	9,80±3,22*	89,1±33,6*	6,10±2,09*	50,7±25,5	0,50±0,27*	312,7±49,8	457,5±56,5	325,3±29,0
G-13_1	17,6±3,11	188,8±60,4	9,40±1,36	237,9±54,7	0,70±0,21	521,3±85,6	509,0±0,00	282,5±11,8
G-13_4	17,1±3,1	26,0±9,0	11,4±2,1	53,9±16,0	0,90±0,17*	278,8±53,0	403,5±29,1	307,7±23,2
G-18_1	12,5±2,54	117,5±34,1	5,20±1,17	201,5±43,7	0,60±0,22	288,4±30,4	562,0±34,5	327,2±17,8
G-18_4	28,9±3,76*	16,9±3,30*	15,2±2,19*	69,1±15,3	1,07±0,16*	525,8±22,2*	-	348,8±26,9
M-10_1	18,3±3,57	27,8±3,44	11,0±2,0	58,5±10,2	0,89±0,26	512,7±88,1	509,5±49,5	328,2±44,4
M-10_4	17,8±3,90	13,4±4,97*	10,0±2,31	46,1±9,72	1,13±0,23*	571,6±88,4*	479,5±36,5*	296,3±36,2
M-13_1	13,6±3,54	64,7±17,5	7,50±1,95	91,3±16,9	1,13±0,30	310,5±44,4	461,0±55,2	336,3±38,4
M-13_4	12,3±2,16	19,3±3,98	5,00±0,96*	69,7±5,66	1,50±0,27*	227,7±36,4	453,8±21,4*	345,6±20,8
M-18_1	6,70±1,84	123,0±26,6	4,00±1,23	165,2±33,2	1,00±0,30	223,3±29,5	428,0±45,8	318,7±33,6
M-18_4	13,9±4,2	18,4±3,62	6,8±1,83*	53,8±5,48	1,8±0,25	252,4±56,5	436,5±30,2	278,2±19,6

\*-p < 0.05 compared with the control group.

Abbr : n- number; sec- second of time.

Table 1. Parameters of sexual behavior in the mature male rats subjected to prenatal exposure to ganglerone (G) or methylbenactyzine (M) at different periods of prenatal development compared with the control group. (M±m).

We believe that sexual dysfunction in adult offspring induced by prenatal exposure to cholinolytics is due to changes to neuronal and endocrine mechanisms. The mechanisms regulating sexual behavior are known to be mediated to a significant extent by neuronal structures located in the preoptic zone of the hypothalamus and to be activated by different neurotransmitter systems, including the cholinergic system (Dorner, 1989). Cholinergic activation of the preoptic area via M1 muscarinic receptors is critical for normal coitus (Hull et al., 1988a; Hull et al., 1988b; Retana et al., 1993). The absence of long-term sexual



dysfunction in offspring subjected to prenatal exposure to methylbenactyzine provides evidence that impairments to sexual function in males are not mediated by the M-cholinergic system of the brain.

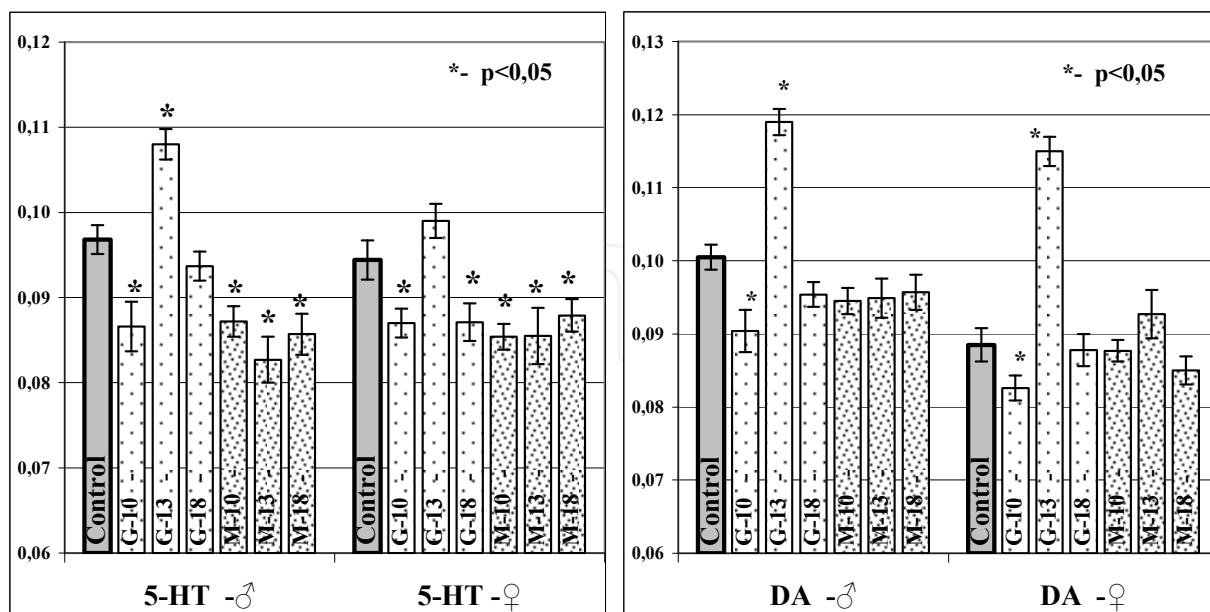
#### 4. Neurochemical subsequences of prenatal exposure of selective M- and N-cholinoblockers in the rat fetus brain on the 20<sup>th</sup> day of pregnancy

Prenatal cholinergic drug exposure to pregnant females resulted in sex-linked alterations of the brain dopaminergic and serotonergic systems. Whereas the brain dopaminergic system of genotypical males and females embryos was more sensitive to influence of N-cholinotropic drug ganglerone.

Neurochemical data analysis of the neurotransmitter status of the rats embryos brain of a various genetical sex has shown that prenatal influence by cholinolytics of the central action - methylbenactyzine and ganglerone- in case of injection on 9-19 days of the gestation causes a disbalance in the content of neurotransmitters of DA, 5-HT and their metabolites in the embryos brain of experimental groups by 20 day of prenatal development in comparison with control.

##### 4.1 Metabolism of dopamine

Males embryos have a weaker density of DA (fig. 3) after prenatal ganglerone exposure on 9-11 days of prenatal development (G10 group). Level of DOPAC (dihydroxy-phenyl acetic acid), DA metabolite, did not change (tab. 2) during the same period. In G10 group decrease of the DA content was accompanied by augmentation of its turnover. At ganglerone introduction on 12-14 days of gestation was noted a substantial growth of DA level (on 29,9 %,  $p < 0,01$ ) which was not accompanied by change of its turnover that testifies the augmentation of mediatory synthesis intensity.



\*- $p < 0,05$  compared with the control group. For further details see caption to Fig. 1.

Fig. 3. Content of dopamine and serotonin (ng/mg of wet tissue) in the Brains of 20-day rat embryos. Notations: - ♂, - ♀ - signs of genotypical males and females embryos.

Females embryos in groups with prenatal ganglerone exposure on 9-11 days of prenatal development also had authentic decrease of DA concentration and its useful increase (on 18,4 %,  $p \leq 0,01$ ) after drug introduction on 12-14 day of gestation. At the same time females had more significant change of DOPAC content. It was also noticed that in contrast to males, females embryos change of DA level was accompanied by more appreciable decrease of DA turnover in all experimental groups. The most appreciable decrease of DA/DOPAC ratio was revealed during early period of gestation after ganglerone exposure and during more late pregnancy after methylbenactyzine exposure.

#### 4.2 Metabolism of serotonin

Alteration of 5-HT level in the embryonal brain was more considerable in comparison with DA. Thus in all investigated periods of gestation in comparison with control group authentically significant reduction of 5-HT content in the embryos brain by exposure of methylbenactyzine and ganglerone was noted.

It was noted that males embryos 5-HT concentration in the brain was significantly decreased in all periods of prenatal methylbenactyzine exposure. In the G10-G18 groups significant decrease of 5-HT concentration in the embryos brain is noted at drug introduction on 9-11 and 17-19 days of prenatal development. Dynamics of 5-HIAA (5-hydroxyindoleacetic acid) content (metabolite of 5-HT) was similar to the mediator content, thus noted significant decrease in M10 - M13 and G10 groups.

The comparative analysis of the received data shows that in the prenatal period the serotonergic transmitter system is more sensitive to exposure of cholinolytics than dopaminergic system. 5-HT concentration and its turnover decrease during the second half of gestation with influence of the methylbenactyzine, and the ganglerone. Whereas the brain dopaminergic system of genotypical males and females embryos is more sensitive to influence N-cholinergic antagonist ganglerone.

Many researches show the sensitivity of neurotransmitter system of a developing brain to influence of various drugs and ecological toxicants, possessing cholinergic activity. For example, the neurochemical alterations caused by the prenatal exposition of nicotine, are well studied; it is noticed that prenationally introduced nicotine damages development of the central mechanisms noradrenergic, dopaminergic, serotonergic and cholinergic system in the rats brain (Lichtensteiger et al., 1988; Ribary & Lichtensteiger, 1989; King et al., 1991; Lichtensteiger & Schlumpf, 1993; Muneoka et al., 1997). Moreover, there is data that various reactions to prenatal exposure of nicotine are bound to a genetical sex of embryos (Genedani et al., 1983; Levin et al., 1993; Shacka et al., 1997).

Thus, prenatal cholinergic drug exposure produced dramatic imbalance of the neurotransmitter contents and turnover in the rat fetus brain on the 20<sup>th</sup> day of pregnancy. The comparative analysis showed that the serotonergic neurotransmitter system was more sensitive to influence of cholinolytics in prenatal period than dopaminergic system. Decreasing of 5-HT concentration and its turnover in all «critical periods» on the second half of pregnancy was marked under influence as methylbenactyzine, and ganglerone, the M- and N-cholinolytics respectively. Whereas the brain dopaminergic system of genotypical males and females embryos was more sensitive to influence of N-cholinotropic drug ganglerone. Thus, prenatal influence of cholinotropic drugs on pregnant females resulted in sex-linked alterations of brain dopaminergic and serotonergic systems at 20-day's old fetuses of rats. These alterations can be involved to ethiopathogenesis of behavioral dysfunctions of rats progenies in pubertal period and be connected with deviant behavior.

groups	n	DOPAC -♂	5-HIAA -♂	DOPAC -♀	5-HIAA -♀
Control	63	0,0121+0,0008	0,1262+0,0030	0,0150+0,0011	0,1292+0,0031
G10	33	0,0132+0,0030	0,1139+0,0032	0,0054+0,0006*	0,1049+0,0034*
G13	16	0,0110+0,0013	0,1390+0,0042	0,0090+0,0009*	0,1280+0,0045
G18	36	0,0054+0,0004*	0,1278+0,0026	0,0102+0,0006*	0,1112+0,0019
M10	41	0,0085+0,0008*	0,1156+0,0028	0,0115+0,0008*	0,1116+0,0030
M13	47	0,0119+0,0004	0,1000+0,0025*	0,0097+0,0005*	0,1034+0,0030*
M18	32	0,0090+0,0009*	0,1061+0,0032	0,0051+0,0003*	0,1074+0,0025*

\*-p < 0.05 compared with control group.

Table 2. Content of DOPAC and 5-HIAA in the brains of 20-day rat embryos. (M ± m)  
Notations: - ♂, - ♀ - symbols of the genetical sex of offspring, accordingly males and females.

## 5. The long-term neurochemical effects of prenatal exposure to selective M- and N-cholinolytics

Prenatal exposure of some neurotropic agents results not only in disturbances of a proliferation and a differentiation of embryos brain neurones, but also causes the remote disorders of development of synaptic function of brain neurones, disturbance of ontogenetic development of the brain basic neurotransmitter systems in the postnatal period (Robinson, 2000; Icenogle, 2004). Studying of development of the central monoaminergic systems of the brain structures participating in regulation neuroendocrinal and behavioral functions of organism of the rats offspring aged 2 months, therefore is obviously important.

The investigations showed that the prenatal exposure of M- and N-cholinolytics to pregnant females produces long-term neurochemical changes in development of brain neuromediator systems. In the investigated of brain structures, both at males, and at females of rats progenies, exposure to prenatal cholinolytics, significant decrease of DA, 5-HT, NA concentrations and change of level of their metabolites is marked.

The analysis of the received data has shown that prenatal introduction of cholinolytics with selective M- and N-cholinergic activity (methylbenactyzine and ganglerone) to pregnant females leads to the remote changes of brain monoaminergic system activity (DA, NA and 5-HT) of 2-month-old rats offspring.

### 5.1 Hippocampus

Rats offspring that were subjected to prenatal exposure of a ganglerone had a decreased in 1,5 - 2,5 times ( $p \leq 0,001$ ) DA level in the hippocampus. The greatest falling of DA level noted

in group with ganglerone exposure on 9-11 days of a gestation (fig. 4). Though in groups with prenatal exposure of a methylbenactyzine DA content in a hippocampus has not changed. In group M10 among offsprings with a prenatal exposition of a methylbenactyzine was noted a tendency to augmentation.

Dynamics of DOPAC in the studied groups in comparison to control groups was opposite to DA content - substantial growth of DOPAC concentration in groups G10 and G13 (accordingly on 21,7 % and 26,3 %,  $p \leq 0,001$ ) was noted. The neurochemical status in hippocampus of the males prenatally exposed to cholinolytics was characterized by serious decrease of DA concentration and change of its metabolite level in comparison with control offsprings.

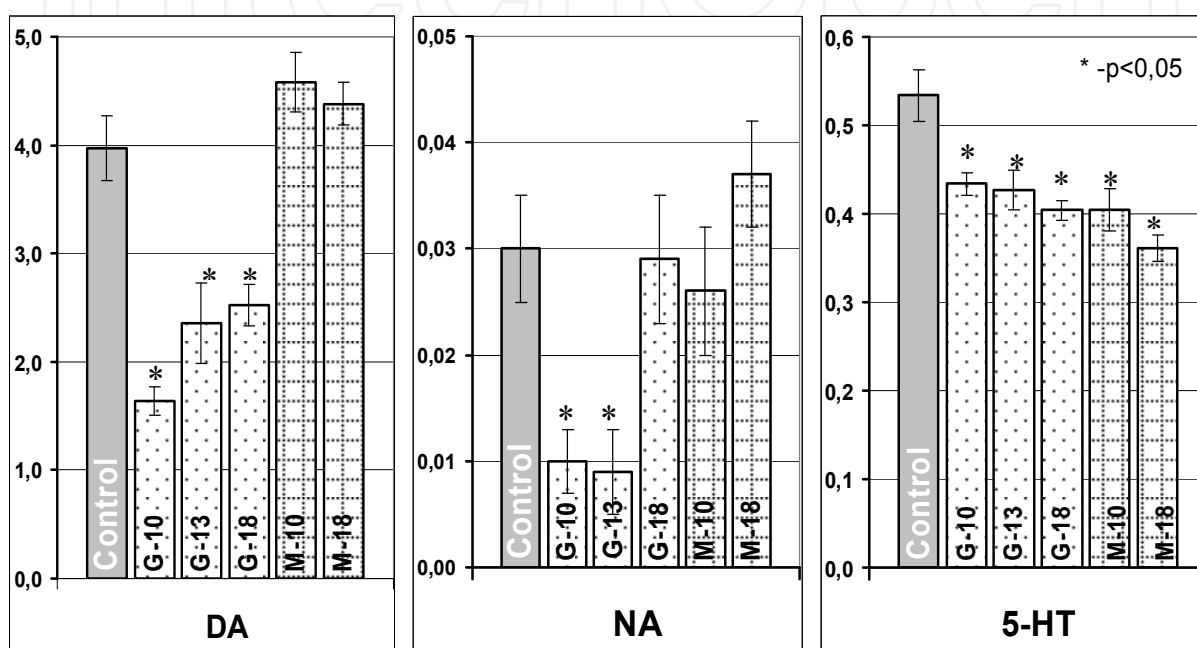


Fig. 4. Contents of dopamine (DA), noradrenaline (NA), and serotonin (5-HT) (ng/mg of wet tissue) in the hippocampus in two-month-old rat offspring exposed to methylbenactyzine or ganglerone at different periods of prenatal development. \* $p < 0,05$  compared with the control group. For further details see caption to Fig. 1.

### 5.1.1 NA metabolism

The obtained data showed decrease of the NA content and increase of its metabolite MHPG (3-methoxy-4-hydroxyphenylethylene glycol) concentration that led to NA synaptic activity decrease. In case of rats males decrease of NA concentration in hippocampus was noted only in two groups subjected to prenatal exposure of a ganglerone in early periods of gestation - G10 and G13 (accordingly 66,6 % and 70,0 %,  $p \leq 0,001$ ). Thus the content of a noradrenaline metabolite of MHPG has been enlarged in all investigated groups in 1,5 - 2,0 times.

### 5.1.2 5-HT metabolism

Unlike other neurotransmitters 5-HT level in the hippocampus has been reduced in all groups of both gender in comparison with control offsprings group. Males offsprings had significant reduction of 5-HT content in the range from 19,7 % ( $p \leq 0,01$ ) - in G10 and to 32,6 % ( $p \leq 0,001$ ) in M18 group. The serotonin metabolite level 5-HIAA in the hippocampus also

has been reduced in groups with prenatal exposure of methylbenactyzine and ganglerone. Ratio indexes 5-HIAA/5-HT have been increased only in those groups in which decrease of mediator level became perceptible.

groups	HIPPOCAMPUS		HYPOTHALAMUS	
	DA	5-HT	DA	5-HT
<b>Control</b>	0,244±0,018	0,976±0,068	0,637±0,037	0,608±0,035
<b>G-10</b>	0,228±0,026	1,120±0,061	0,470±0,023*	0,578±0,041
<b>G-13</b>	0,194±0,022	1,192±0,078*	0,461±0,030*	0,636±0,028
<b>G-18</b>	0,340±0,020*	1,134±0,062*	0,540±0,026	0,587±0,032
<b>M-10</b>	0,344±0,022*	1,240±0,059*	0,378±0,033*	0,538±0,039
<b>M-18</b>	0,402±0,031*	1,188±0,069*	0,490±0,041*	0,550±0,039

Table 3. Turnover of DA and 5-HT neurotransmitters in hippocampus and hypothalamus at 2-month-old rat offspring exposed to methylbenactyzine or ganglerone at different periods of prenatal development. (M ± m) \**p* < 0.05 compared with the control group

Research of neurotransmitters turnover in the hippocampus has shown that rats offspring subjected to exposure of cholinolytics have an enhancement of the turnover of the basic mediators, leading to decrease of concentration of these neurotransmitters in the hippocampus. Noted increase of DA turnover in the hippocampus of rats offspring prenatally subjected to exposure by methylbenactyzine (tab. 3) and significant increase of 5-HT turnover in the hippocampus of the offsprings, subjected to prenatal exposure both methylbenactyzine and ganglerone.

## 5.2 Hypothalamus

### 5.2.1 DA metabolism

The neurochemical status of DA in the hypothalamus of 2-month-old rats offsprings subjected to prenatal exposure of cholinolytics, was characterised by significant decrease of dopaminergic activity in relation to control (fig. 5). In comparison with the methylbenactyzine, prenatal exposure of the ganglerone, caused more appreciable remote changes of DA level in the hypothalamus of these offsprings.

Concentration of the DA has been reduced in all groups - G10 - G18 (31,7 % - 36,9 %, *p* ≤ 0,001) with maximally low value in G13 group. Among offsprings with prenatal exposure to methylbenactyzine significant decrease of DA noted only in M18 group (17,6 %, *p* ≤ 0,05). Dynamics of DOPAC change in relation to control group was similar to dynamics of DA significant 1,5 - 2 times decrease of DOPAC concentration in all groups was noted.

### 5.2.2 NA metabolism

The neurochemical status of NA in the hypothalamus was characterised by rising of processes of degradation of a mediator without enhancement of synthesis processes. In all studied groups both males and females had a decreased NA level and high MHPG content in the hypothalamus in comparison with control offsprings' group. In comparison with methylbenactyzine, NA concentration in groups with prenatal exposure to ganglerone, has been reduced more considerably (in a greater degree in G10 group - decrease of the NA of 26,2 %,  $p \leq 0,001$ ). The content of MHPG metabolite, on the contrary, has been raised in all groups in 1,5 - 2,5 times.

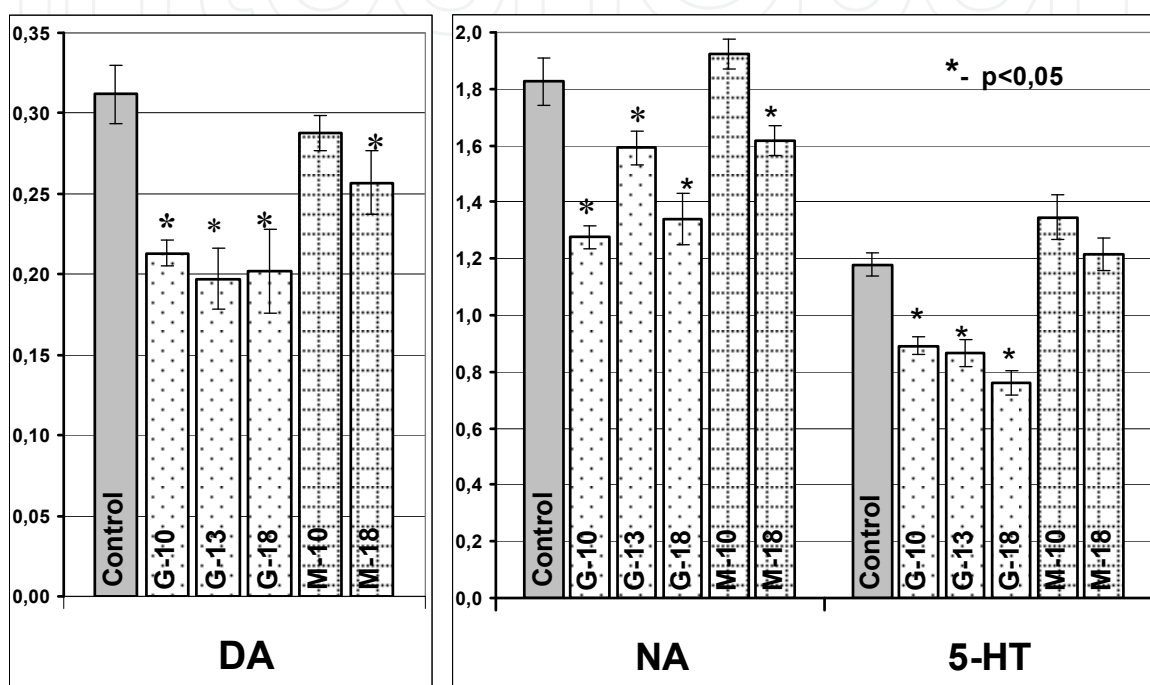


Fig. 5. Contents of dopamine (DA), noradrenaline (NA), and serotonin (5-HT) (ng/mg of wet tissue) in the hypothalamus in two-month-old rat offspring exposed to methylbenactyzine or ganglerone at different periods of prenatal development. \* $p < 0,05$  compared with the control group. For further details see caption to Fig. 1.

### 5.2.3 5-HT metabolism

Dynamics of 5-HT change in the hypothalamus was similar to other mediators - rats offsprings from G10 - G18 groups, where mediator level has been reduced in significant limits (accordingly, 24,3 % - 35,4 %,  $p \leq 0,001$ ), had more radical changes in 5-HT content in G10 - G18 groups. In methylbenactyzine groups 5-HT concentration in the hypothalamus strengthened (in M10 group on 14,2 %,  $p \leq 0,05$ ). 5-HIAA metabolite content in the hypothalamus of all the studied groups was similar to dynamics of the mediator.

The greatest changes of the neurotransmitters turnover in the hypothalamus have been detected concerning a DA both of rats males and females (tab. 1). DA turnover has considerably reduced in the males hypothalamus in all experimental groups in comparison with control group. The ratio index 5-HIAA/5-HT was more stable, except for G10 group of females which index was reduced. In the same group increase of 5-HT level at the stable content 5-HIAA was noticed that shows enhancement of serotonergic synaptic activity in

the hypothalamus. NA turnover in the hypothalamus of experimental groups was comparable with the data of control group though the mediator content and its metabolite has been reduced in all groups.

Sexual dimorphism in effects of prenatal exposure of M- and N-cholinolytics on dopaminergic system (fig.6) has been detected. In case of males offspring that were exposed to prenatal exposure of a ganglerone N-cholinolytics the decrease of DA concentration in the brain structures was noted; in case of rats females - the same reduction after prenatal exposure to M-cholinolytic methylbenactyzine.

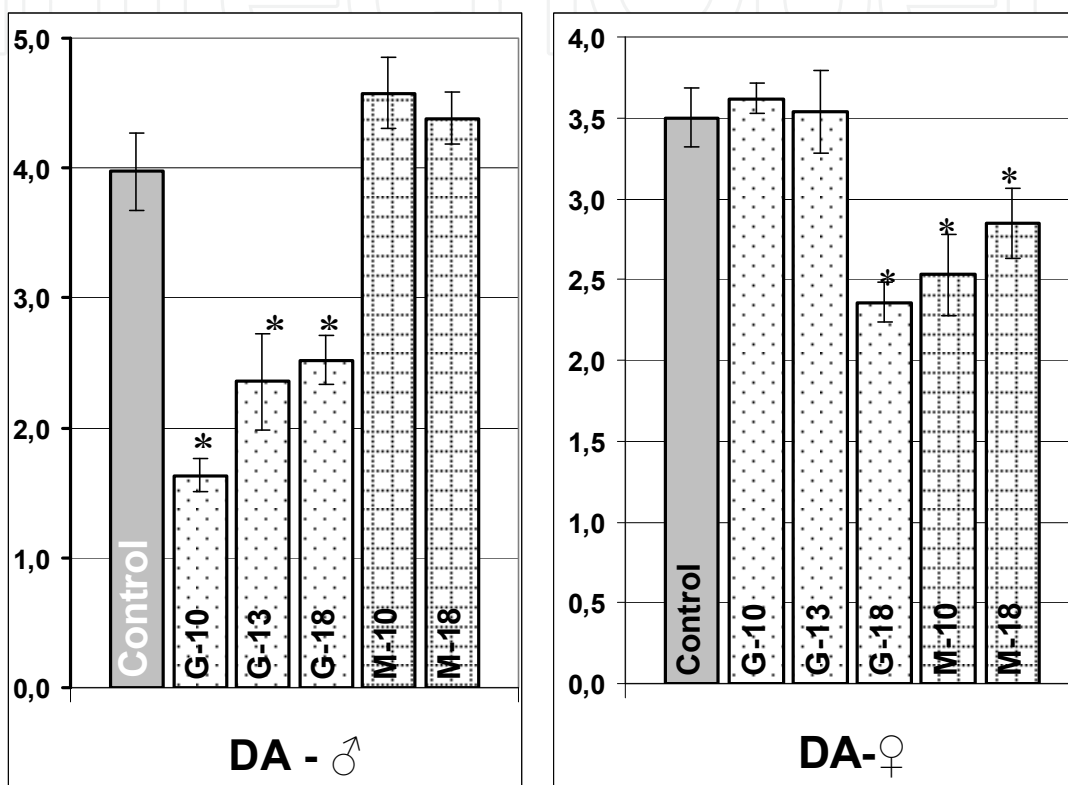


Fig. 6. Sexual dimorphism according to dopamine concentration (ng/mg of wet tissue) in the hippocampus in two-month-old rat offspring exposed to methylbenactyzine or ganglerone at different periods of prenatal development. \* $p < 0.05$  compared with the control group. Notations: - ♂, - ♀ - symbols of the genetical sex of offspring, accordingly males and females.

### 5.3 Amygdala

For realization of adaptive and sexual behavior dopaminergic system of amygdala is also important. The amygdala initiates the organization of adequate behavior to the situation and by means of influence on the hypothalamus and vegetative excitatory system frames conforming hormonal and neurovegetative assurance to this behavior (Simonov, 1987). According to many researchers an amygdaloid complex is responsible for integration of emotional expressions, characteristic for sexual motivation (Newman, 1999; Dominguez, 2001).

Dynamics of neurotransmitters level in the amygdala was similar to the neurochemical status in the hippocampus that once again confirms their morphophysiological generality

within limbic system. Long-term effects of prenatal exposures of cholinolytics within amygdala nuclei have been brightly expressed. Study's results show significant decrease of DA, 5-HT and NA mediators content in the amygdala of the males prenatally subjected to exposure of ganglerone, larger degree on 10-13 days of gestation (fig. 7). Prenatal exposure of M-cholinolytic methylbenactyzine had no strongly pronounced consequences on development of neurotransmitter systems in the amygdala

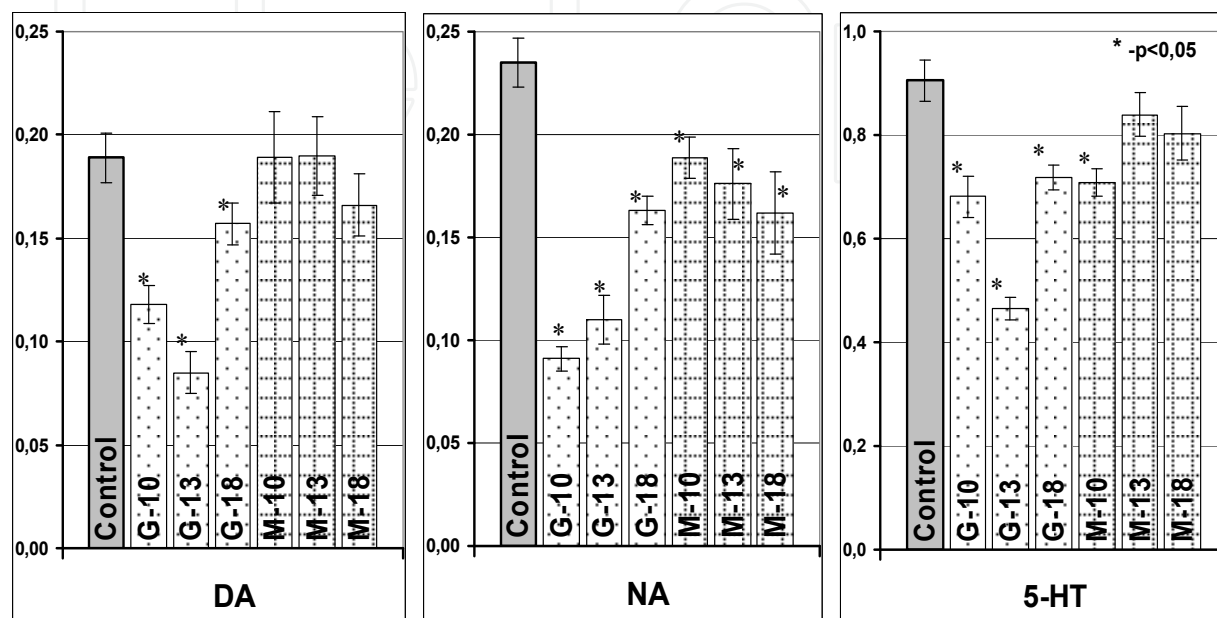


Fig. 7. Contents of dopamine (DA), noradrenaline (NA), and serotonin (5-HT) (ng/mg of wet tissue) in the amygdala in two-month-old rat offspring exposed to methylbenactyzine or ganglerone at different periods of prenatal development. \* $p < 0.05$  compared with the control group. For further details see caption to Fig. 1.

Changes of the neurotransmitter status in the amygdala can affect functioning of other brain structures participating in regulation of sexual function, by means of neuronal connection of this structure with hippocampus and hypothalamus. It is shown that destruction of hypothalamo-amygdaloid connection, damage or irritation of the amygdala lead to neurohumoral alterations and sexual behavior abnormalities (Akmaev, 1993; Swanson, 1998; Dominguez, 2001). It is not excluded that optimization of integrative connections within limbic system and hypothalamus is extremely important concerning the effects of cholinergic system on sexual function.

Results of these researches show that pregnant females in "critical periods" of the embryo prenatal development exposure to M- and N-cholinoblockers can cause long-term changes in neurotransmitter systems activity in investigated structures of the rats brain in their postnatal life.

Exposure of M- and N-cholinolytics in the prenatal period leads to significant decrease of dopaminergic activity in the hippocampus and hypothalamus of rats offspring in comparison with control group. Results of various researches prove that defects of DA synaptic activity in the hippocampus at offspring can accompany to hippocampus-associated behavioral deficiencies at puberal individuals (Yanai, 1984; Smith, et al. 1986; Steingart et al., 2000).



The prenatal nicotine exposure which is exogenous ligand of N-cholinergic receptor leads to nonperishable change of activity of the basic neurotransmitter systems in brain structures in a postnatal period. The decrease of DA concentration in the brain structures at males has been detected at offspring which were subjected to prenatal exposure of N-cholinolytic ganglerone, and at females - to mainly prenatal exposure of M-cholinolytic methylbenactyzine (Genedani et al., 1983; Ribary, 1985; Lichtensteiger et al., 1988; Lichtensteiger & Schlumpf, 1993; Muneoka et al., 1997).

In spite of various mechanisms of M- and N-cholinolytics action in the organism, the long-term effects of these drugs on the basic transmitter systems development in offspring are basically the same, namely inhibiting a metabolism of these neurotransmitters. Prenatal exposure of selective M- and N-cholinolytics, like other chemical drugs and ecological toxicants with cholinergic properties, causes the long-term changes in programming of neurotransmitter functions in 2-month's offspring which, in turn, can participate in the development of neuro-behavioral anomalies, appetent and affective disturbances at puberal individuals (Lauder, 1985; Turlejski, 1996; Levitt et al., 1997; Dreyfus, 1998; Azmitia, 2001).

Thus, the prenatal exposure of M- and N-cholinolytics to pregnant females produces long-term neurochemical changes in the development of brain neuromediatory systems. In the studied brain structures, both males and females, exposure to prenatal cholinolytics, significant decrease of DA, 5-HT, NA concentrations and change of level of their metabolites were marked. Prenatal exposure to ganglerone N-cholinolytics to pregnant females on 9-19 gestational days exerts most appreciable long-term effect on neurotransmitter development, which leads to reduction of dopaminergic, noradrenergic and serotonergic activity in the hippocampus and hypothalamus of 2-month-old rats progenies in comparison with control group. It was noted that prenatal exposure of cholinolytics on DA concentration in the brain structures at 2-month-old rats progenies causes sexual dimorphism. These results indicate that exposure to M- and N-cholinolytics during the critical periods of prenatal development (9-11 and 12-14 gestational days) results in long-term changes in development of neuromediatory systems in the brain structure, which participate in regulation of behavioral and neuroendocrinal functions of rats offsprings.

## **6. Endocrinological consequences of prenatal exposure to N-cholinolytics in the rats offspring's**

The involvement of N-cholinergic mechanisms in behavioral impairments in male offspring is associated with the properties of N-cholinoreceptors, which are involved in the regulation of the catecholaminergic system of the CNS. N-cholinergic neurons are connected to different types of neurons in the brain, and activation of N-cholinoreceptors by endogenous (acetylcholine) or exogenous (nicotine) ligands modulates the release of the transmitters DA, NA, and 5-HT, depending on the type of cell (Retana, 1993; McGehee et al., 1995). Nicotinic receptors are abundant in the subcortical areas of the brain during early fetal development (Lisk & Greemvald, 1983), so embryonic exposure to nicotine damages noradrenergic and dopaminergic synaptic transmission in the brain (Navarro et al., 1988; Seidler et al., 1992).

It follows from this that the mechanism of prenatal exposure to the N-cholinolytic ganglerone on sexual function in offspring may be mediated by modulation of brain transmitter systems, including DA, NA, and 5-HT systems, which indirectly regulate the processes of motivation and components of coitus (Bitran & Hull, 1987; Gladkova, 2000; Mas et al., 1987; Pfaus & Phillips, 1991). This thesis is supported by results from neurochemical

studies, which have demonstrated that administration of an N-cholinolytic to pregnant females at different periods of gestation leads to long-term changes in the development of brain neurotransmitter systems in 20-day embryos and two-month offspring of rats.

The most significant changes in the concentrations of DA, 5-HT, and NA and their metabolites in brain structures were seen after administration of ganglerone at 9–11 and 12–14 days of gestation, which was apparent as reductions in the synaptic activities of these brain neurotransmitter systems, particularly dopaminergic activity in the hippocampus and hypothalamus in two-month-old offspring of rats as compared with controls. Although 20-day embryos showed an increase in DA levels in response to administration of ganglerone at 12–14 days of gestation, this requires further investigation. It can be suggested that like nicotine, ganglerone, blocking N-cholinergic receptors, induced impairments to the formation and establishment of network systems in the developing embryonic brain, which promoted stable decreases in the synaptic activity of the transmitter systems of interest in the hippocampus and hypothalamus of twomonth-old offspring rats as compared with controls. This imbalance in neurotransmitter activity in brain limbic structures in two-month-old offspring is a long-term neurochemical effect of prenatal treatment with ganglerone, which in turn may facilitate sexual dysfunction in fertile males.

The neurotransmitter DA plays an important role in activating the sexual behavior in male rats; DA depletion in brain structures involved in regulating the behavioral states of body facilitates reductions in sexual activity (Gladkova, 2000; Mas et al., 1987; Pfaus & Phillips, 1991). Lesioning of different dopaminergic projections induces different behavioral syndromes depending on which part of the CNS is lesioned (Carey & Schwarting, 1986; Simon et al., 1986); in particular, damage to the dopaminergic projections of limbic structures leads to impairments in male sexual function (Hull et al., 1984).

The noradrenergic and serotonergic systems of the brain are also involved in regulating hormone-dependent behavioral states, including sexual behavior (Naumenko et al., 1983; Lenahan et al., 1986; Smeets & Reiner, 1994). Affecting the secretion of gonadoliberein in the hypothalamic nuclei, NA and 5-HT act on  $\alpha_2, \beta_2$ -adrenoreceptors and 5-HT<sub>1,2</sub> serotonin receptors to take part in the central regulation of the endocrine system of the male body and, thus, in controlling male sexual function.

Thus, prenatal modulation of N-cholinergic brain mechanisms with ganglerone can alter the activities of DA, NA, and 5-HT systems, which are directly involved in regulating motivation and components of coitus in adult offspring. We believe that this mechanism is the main cause of long-term behavioral impairments in pubescent offspring subjected to prenatal exposure to cholinolytics. The prenatal effects of cholinolytics on sexual function in offspring represent a paradox, which is that in relation to the cholinergic system, sexual activity in adult males is regulated mainly by M-cholinolytic mechanisms, while during the prenatal period, these are more dependent on the activity of N-cholinergic system.

Another mechanism mediating the long-term actions of cholinolytics on sexual function in offspring consists of the involvement of endocrine factors. Considering the role of the central and peripheral compartments of the nervous system in controlling the hypothalamo-hypophyseal-gonadal system in males, it can be suggested that prenatal administration of cholinolytics to pregnant females might also have long-term consequences in relation to the endocrine system of offspring.

The results obtained from endocrine studies supported the occurrence of endocrine impairments in pubescent offspring (Fig. 8). A significant reduction in testosterone levels was seen in offspring subjected to prenatal exposure to ganglerone at different periods of

gestation, with lowest values seen in the offspring of group G13 (2.4-fold decrease). There was also a significant decrease in the testosterone level in offspring subjected to prenatal exposure to methylbenactyzine in group M18. The LH and FSH levels in the blood of all groups were increased.

The hormonal-motivational component of sexual behavior of male rats is known to be controlled at the central level by testosterone, which is metabolized to estradiol, while ejaculation is controlled at the peripheral level by the non-aromatized dihydrotestosterone and only partially by testosterone (Lisk, 1983). Low testosterone levels in G10–G13 offspring could therefore facilitate alterations in both the central motivational and the peripheral ejaculatory components of sexual behavior. The reduced testosterone level in G10–G18 offspring correlated with low sexual activity and, conversely, numbers of the sexually more active males of groups M10–M18 had higher testosterone levels.

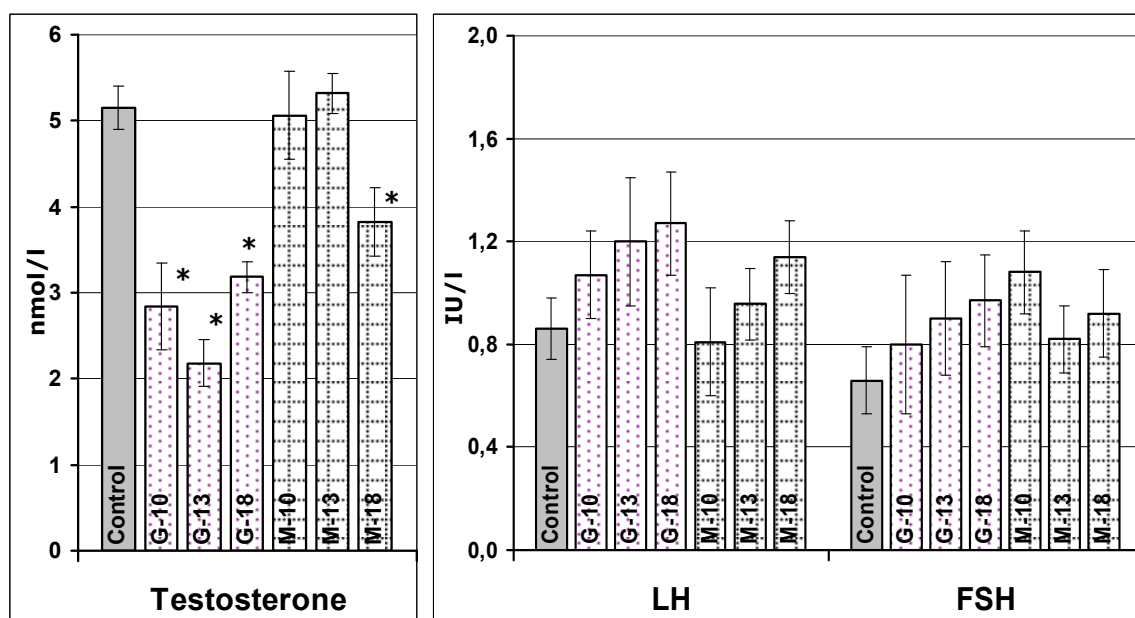


Fig. 8. Serum Testosterone, LH and FSH levels in two-month-old rat offspring exposed to ganglerone or methylbenactyzine at different periods of prenatal development. \* $p < 0.05$  compared with control group. For further details see caption to Fig. 1.

Thus, along with neuronal factors, changes in the hormonal background probably represent a further cause of impairments to sexual functions in offspring subjected to prenatal exposure to central cholinolytics. Reproductive impairments induced by damage to the neuroendocrine and neurotransmitter systems during the fetal period of ontogenesis due to prenatal exposure to cholinolytics may in later life become the cause of impairments to the ability of males to mate and produce offspring.

## 7. Correction of sexual dysfunction of the males subjected to influence of selective cholinolytics in the early prenatal period

The studies showed that rat males characterized by low sexual activity, were very sensitive to effects of agonists of the cholinergic and dopaminergic systems. The correction of sexual activity was observed only during the period of action of these drugs and did not appear in delayed period after treatment.

Experimental researches have shown that the rats males subjected to prenatal exposure of a ganglerone and characterized by low sexual activity, have appeared sensitive to effects of cholinergic and dopaminergic (tab. 4).

G13 group		Mounts (n)	latency of mount (sec)	Intromissions (n)	latency of intromission (sec)	Ejaculation (n)	latency of ejaculation (sec)	Interejaculatory interval (sec)
Control		17,1 ±3,1	26,0 ±9,0	11,4 ±2,1	53,9 ±16,0	0,90 ±0,17	278,8 ±53,0	403,5 ±29,1
Arecoline	1 hour	29,6 ±1,3*	13,3 ±2,0*	17,1 ±2,9*	29,5 ±3,2*	1,83 ±0,22*	184,4 ±36,4*	313,4 ±41,2*
	7 days	14,1 ±2,1	21,9 ± 3,8	10,1 ±2,9	42,3 ±7,8	1,04 ±0,18	267,0 ±41,5	394,1 ±54,3
Galantamine + Ganglerone	1 hour	24,6 ±2,8*	12,4 ±2,5*	18,8 ±3,1*	28,7 ±4,7*	1,55 ±0,19*	198,3 ±36,0*	329,1 ±39,5*
	7 days	16,6 ±2,1	30,5 ± 4,1	12,0 ± 2,4	55,8 ±7,2	1,10 ±0,17	288,2 ±41,5	386,0 ±50,6
Apomorphinum	1 hour	31,6 ±4,4*	8,30 ±2,3*	21,2 ±3,8*	23,3 ±5,9*	1,97 ±0,30*	166,2 ±32,1*	320,5 ±52,6*
	7 days	19,9 ±3,0	24,2 ± 3,6	12,2 ±1,2	45,4 ±6,7	1,09 ±0,18	237,3 ±38,2	425,0 ±57,9

\*-p < 0.05 compared with control group.

Note: The sexual activity is recorded in 1 hour after an injection and for 7 days of an afteraction of drugs.

Table 4. Parameters of sexual behavior in the mature male rats subjected to prenatal exposure to ganglerone on 12-14 day of gestation (G13 group) before and after application of the agents. (M±m).

Cholinomimetic drug arecoline (2 mg/kg), galantamine with a ganglerone (accordingly, 1mg/kg and 5 mg/kg) and agonist of D<sub>1</sub>,D<sub>2</sub>-dopaminergic receptors apomorphinum (1 mg/kg) considerably enhanced sexual function. Components of sexual function - mounts, intromissions and ejaculations after pharmacological correction were high enough though did not reach in certain cases indexes of sexual behavior of control offspring. On the contrary, time components of a sexual behavior specified about sufficient high degree of sexual activation, including motivation enhancement.

In spite of significant enhancement of the sexual function, the obtained data have shown that correction of sexual activity descended only during the period of drugs action - within 1 days. For 7 day after introduction of stimulating drugs quantitative and qualitative characteristics of rats male sexual behavior were reverted on initial level.

Prenatal exposure of cholinolytics also promoted appearance of high sensitivity of sexual function of offspring to effects of antagonists cholinergic and dopaminergic systems. The methylbenactyzine (3 mg/kg) and haloperidolum (0,5 mg/kg) in the doses depressing sexual activity of intact rats only to 50 %, completely quenched implication of sexual function at offspring G10, G13 and M10 groups.

Thus, the research results show that sexual dysfunction of the offspring subjected to prenatal exposure of M- and N-cholinolytics is a persistent sexual function abnormality that demands long courses of pathogenetic treatment.

## 8. Conclusions

- Results of the investigations show that prenatal exposure by ganglerone and methylbenactyzine leads to the delayed behavioral disturbances, significant and stable failure of sexual function of puberal males offspring. Ganglerone administration to pregnant females on 10-13 days of gestation has led to violent decrease of sexual function at puberal males, low level of ejaculatory components of sexual behavior with long enough stage of ejaculation latency. Significant damage of the motivational component, high latence of mount and intromissions of offspring in G10 and G13 groups was detected. Change of sexual activity of offspring with methylbenactyzine exposure have been less expressed, and after acquisition of sexual experience, these changes in comparison with control were levelled.
- The certain paradox in effect of cholinergic drugs on sexual function of males was noted: sexual activity of males is regulated by M-cholinergic system and prenatally depends on activity of N-cholinergic system. Neurotransmitter dysfunction of fertile 2-month-old males that were prenatally administered with cholinolytics predetermines behavioural disturbances, in particular sexual dysfunction of puberal offspring.
- Analysis of the received neurochemical data of the brain neurotransmitter status of 20-day-old embryos of a various genetical sex have shown that prenatal administration of cholinergic drugs of the central action type (methylbenactyzine and ganglerone) in different periods of gestation, causes a disbalance of the content of DA and 5-HT neurotransmitters and their metabolites in the embryos brain on 20 day of prenatal development in comparison with control group.
- Results of the experiments show that modulation of activity by M-cholinergic and N-cholinergic systems of a developing foetus brain can lead to significant changes in activity of the basic transmitter systems of an embryonal brain. Accordingly, mechanisms of prenatal exposure of various chemical factors with cholinergic properties can be mediated both M-cholinergic and N-cholinergic system.
- In the prenatal period the serotonergic transmitter system is more sensitive to exposure of cholinolytics than dopaminergic system. The serotonergic transmitter system is more sensitive to exposure of methylbenactyzine and ganglerone. The brain dopaminergic system of genotypical males and females embryos is more sensitive to N-cholinolytic ganglerone exposure.
- Exposure of pregnant females in "critical periods" of prenatal embryo development to M- and N-cholinoblockers caused long-term changes in activity of neurotransmitter systems in brain structures of 2-month-old rats offspring. Significant decrease of DA, 5-HT, NA concentration and change of level of their metabolites in the brain structures

participating in regulation of behavioral and neuroendocrinal functions of organism was detected.

- The most significant effect of ganglerone administration on neurotransmitter development was noted on 10-13 days of gestation when it led to decrease of synaptic activity of transmitter systems and growth of dopaminergic activity in the hippocampus and hypothalamus of 2-month-old rats offspring in comparison with control group.
- Change of hormonal background and significant decrease of the testosterone level in comparison with control offsprings' group is one of the causes of the reduced sexual function at the offspring subjected to prenatal ganglerone exposure. Low level of testosterone correlated with low sexual activity and high quantity of sexually inactive males in the same groups.
- Pharmacological correction of the reduced sexual activity descends only during a period of action of stimulating drugs. For 7 days after administration of stimulating drugs quantitative and qualitative characteristics of sexuality of rats males were reverted on basic level.

Thus, administration of cholinergic drugs to rats in the prenatal period produces prolonged influence on the neurotransmitters level, sexual hormones and sexual activity in adulthood. The reproductive problems caused by injuries of neuroendocrine system during the fetal period can compromise the later success of mating as well as the capacity to generate descendants.

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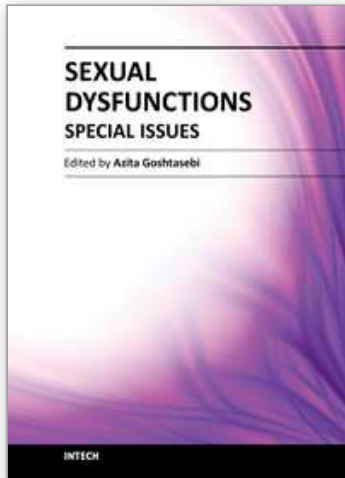
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