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*Chapter II*

# Modulation of the Dentate Area's Postnatal Neurogenesis in Rats and Mice: Behavioral Consequences and the Role of Genotypes

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## Abstract

The developing brain is able to respond to numerous external stimulations, most of them being necessary for normal development. Although, in the array of different agents which that could induce changes in the course of developmental processes are those which may be alien to normal developmental processes. Pharmacological agents are described which are capable to modulate the course of normal postnatal neurogenesis in rodents. The remote effects of these neonatal injections (the changes in behavior and/or physiological reactions in adult animals) are the goals of many studies during the last ten years, the interest to and practical importance of such data gradually increasing. The latter means that artificial interference to the normal brain ontogeny could modify the CNS development in such a way that the consequences could be detected in functions of the adult brain. The modulation of the postnatal neurogenesis, which includes the possibility to intensify this process, could be a plausible strategy for a brain development study as well as a way to investigate regeneration processes in the CNS. It signifies the importance of studying the behavioral and morphological changes as the consequences of treatments influencing the postnatal neurogenesis.

Injections of L-NG-nitroarginine methyl ester (L-NAME), the inhibitor of NO-syntase, and of semax (the synthetic analogue of ACTH 4-10) to rat and mouse pups of definite genotypes during first three weeks of life were performed. The Wistar rats and rats of the audiogenic seizure prone Krushinsky-Molodkina strain were used as well as

mice of 101/HY and C3H/He strains. It was demonstrated that these treatments significantly increased the number of proliferating cells in the dentate area ( particularly the number of neuronal precursors), and this effect persisted after the injections were finished. This remote effect was more intense in cases of semax injections. These treatments were accompanied by changes in the anxiety behavior of these animals as adults and were genotype dependent. The remote effects of neonatal L-NAME and semax were revealed in an increase of successful elementary logic cognitive task solutions (the capacity to extrapolate the direction of food bait movement when it disappears from the animal's view). These neonatal injections induced changes in audiogenic seizure proneness as well.

A new aspect associated with changes induced in a developing brain is presented, underlining the fact that they could be at least partly related to an increase in the intensity of postnatal proliferative activity in the brain.

**Keywords:** Adult neurogenesis, neonatal age, rats, mice, audiogenic seizures, locomotion, anxiety, exploration, cognitive task, genotypes

# Introduction

The process of adult brain neurogenesis is one of the most intriguing processes in the brain, and it was investigated rather extensively during last 15 years. This process is not rigid; nonetheless, it could be modulated, and it could play a certain role in the mechanisms that are involved in the realization of brain plasticity (Lledo et al., 2006). Laboratory experiments revealed a set of factors, environmental enrichment (Kempermann et al., 2002; Ra et al., 2002; Auvergne et al., 2002; Brown et al, 2003; Rizzi et al., 2011) and isolation from conspecifics (Spritzer et al., 2011) being among them, which could modulate brain postnatal neurogenesis (Kempermann, 2002; Ehninger, Kempermann, 2008; Bonfanti, Peretto, 2011;. Neurotrophins (e.g. BDNF) and other endogenous molecules (Wagner et al., 1999; Xiong et al., 1999; Bagni et al., 2002; Rossi et al., 2006; Dolotov et al., 2006; Henry et al., 2007; Thakker-Varia et al., 2007; Bath and Lee, 2010; Bekinschtein et al., 2011) and NO production (Estrada et al., 1997; Packer et al., 2003; Moreno-Lopez et al., 2004; Zhu et al., 2006) also participate in the complicated chain of processes underlying neurogenesis. Furthermore, species and age differences were noted in adult neurogenesis (Gould et al., 1997; Amrein et al., 2004). The hormonal status of the organism, genotype and behavior are also factors (Kempermann et al., 1997; Kempermann, Gage, 2002 a, b; Almgren et al., 2007; Perfilieva et al., 2001; Hayes, Nowakowski, 2002). Adult neurogenesis has been found in all mammals studied to date, including humans (Kempermann et al., 2004; Ehninger, Kempermann, 2008; Bonfanti, Peretto, 2011).

The modulation of postnatal brain neurogenesis is one of the plausible ways to study both the developmental processes in the CNS and the regenerative capacities of developing or adult brains.

In the array of different agents which could induce changes in the course of brain developmental processes, there are those which may be alien to normal developmental processes (Frischer et al., 1988; Rodier, 1995; Ojima et al., 1996; Ferguson et al., 2001; Salonin et al., 2004; Olczak et al., 2010; Walker et al., 2010; Niculescu et al., 2011; Hays et al., 2012). There are many pharmacological agents that are capable to modulate the course of

normal postnatal neurogenesis in rodents that are being introduced to animals during their early-postnatal period or prenatally. The problem associated with modulating effects, which certain drugs could exert on adult behavior when neonatally introduced, is still waiting on a thorough analysis, although they were already described for several SSRI and peptide agents (the synthetic analogue of the ACTH 4-10 fragment, in particular - semax) (Dawirs et al., 1998; Malberg et al., 2000; Boyarshinova et al., 2004; Shilova et al., 2004; Santarelli et al., 2003; Encinas et al., 2006). Creating the remote effects of neonatal injections (the changes in behavior and/or in physiological reactions in adult animals) was the goal of many studies during recent years. The latter means that artificial interference with the normal ontogeny could modify brain development in such a way that the consequences could be detected in adult brain functioning (e.g. Angelucci et al., 1999). The theoretical and practical importance of such an approach is due to the possibility of investigating the plausible deviations of normal brain development. This, in turn, would help to understand normal development in greater detail and to study the new possibilities which could arise in the field of nervous system regeneration processes. This approach could initiate the thinking associated with the early treatment of CNS disfunctionsdysfunctions (even of genetic origin) by analyzing the long-term consequences of various neonatal treatments (Silva, Ehninger, 2009; Bianchi et al., 2010). In general, it is important to elucidate the possible consequences of early treatment by agents which are capable to induce changes in the neurogenesis.

The proliferative activity in the adult mammalian brain is characteristic for two main forebrain areas – the dentate gyrus area of hippocampal formation and the superventricular forebrain zone (SVZ) (Kempermann et al., 2003). Changes of cell division intensity during the early postnatal period could be crucial not only because additional cells appear in the respective brain structure, but also because of there is an increase in the expression of neurotrophic factors, proceeding in parallel (Maisonpierre et al., 1990; Hodes et al., 2010; Bath and Lee, 2010, et al.).

We used two chemical agents – the first one was semax (russianRussian abbreviation for "seven aminoacidsamino acids"). Semax is the synthetic analogue of ACTH fragment 4-10, the initial amino acid sequence of the native peptide being modified in order to prevent the cleavage of the molecule by endopeptidases (Ashmarin et al., 2003; Levitskaya et al., 2004; Zolotarev et al., 2006; Agapova et al., 2007). Another agent used was L-NAME (N-nitro-L-arginine methyl ester), an inhibitor of NO-syntase (NOS). It was demonstrated that NOS inhibition is accompanied by an increase of adult brain neurogenesis (Estrada et al., 1997; Xiong et al., 1999; Packer et al., 2003; Matarredona et al., 2004, 2005; Fritzen et al., 2007). The data presented in this chapter concerns the postnatal neurogenesis in a subgranular layer of the hippocampal dentate gyrus, shown to produce new neurons over an anima's lifelife (Mackowiak et al., 2004). Experiments were performed in laboratory rats and mice of different genotypes. The evaluation of the remote effects from early treatments has animportantan important genetic aspect: the direction and scope of the remote behavioral deviation from controls was different in different strains of rats and mice (Vorhees et al., 1999; Shilova et al., 2004; Boyarshinova et al., 2004 a, b).

This chapter contains experimental data, demonstrating the remote effects of early treatments associated with changes in the cell proliferation process. The behavioral investigations included: a test of audiogenic seizure proneness; an open field test, which estimates locomotion levels; an elevated plus maze test, whichevaluateswhich evaluates the

level of animal anxiety and exploration (Roohbakhsh et al., 2007); and an extrapolation elementary logic task (extrapolation task), which belongs to the category of tests which evaluates the cognitive abilities of animals, i.e. reaching the test's solution without previous learning (Krushinsky, 1990; Leitinger et al., 1994, Pereplkina et al., 2006).

As mentioned above, two types of neonatal treatments were used, –injections of either neuropeptide semax or L-NAME (N-nitro-L-arginine methyl ester), the inhibitor of nitric oxide synthase (NOS) activity. The neurotrophic and nootropic effects of semax had been demonstrated (Levitskaya et al., 2004; Sebentsova et al., 2005 a,b). The commercial pharmacological form of Semax already exists in Russia. Semax increased the neurogenesis in neural cell cultures (Grivennikov et al., 1999, 2008), whereas no *in vivo* effects from this drug on postnatal neurogenesis had been demonstrated before.

As mentioned above, nitric oxide (NO) controls proliferation processes in the brain, as well as cell migration and differentiation, demonstrated in different experimental models. It was established that NO suppresses the proliferation and induces cells to start the differentiation, although the data concerning the NOS suppression in brain proliferative areas are possibly contradictory (Volke et al., 1997; Moreno et al., 2004; Estrada et al., 1997, 1998; Cheng et al., 2003; Matarredona et al., 2004, 2005; Packer et al., 2003). NO is the important endogenous factor that determines the cell differentiation not only in cell cultures, but *in vivo* as well (Peunova, Enikolopov., 1995); and not only in neuronal precursors, but in cells of other types (Lee et al., 1997; Nisoli et al., 1998; Bloch et al., 1999; Matarredona et al., 2005). In these experimental conditions, NOS-inhibition (by L-NAME introduction) increased adult brain cell proliferation (Virgili et al., 1999; Matarredona et al., 2004; 2005) and had behavioral, mainly anxiogenic, effects (De Oliveira et al., 1997; Del Bel et al., 2000, 2005; Czech et al., 2003; Miguel et al., 2008). The inhibition of NO production is reported to have several negative consequences in the paradigms of instrumental and spatial learning e.g., the negative effects of morphine on memory formation were shown to depend on a decrease of NO synthesis (Khavandgar et al., 2003). The improvement of spatial memory consolidation, resulting from the administration of a pharmacological agent, atorvastatin, was negatively affected by the L-NAME treatment in adult animals (Rayatnia et al., 2011). Simultaneously, it was shown that L-NAME-treated groups did not significantly differ in the acquisition and retention of spatial and cued tasks (Knepper and Kurylo, 1998). Thus, in contrast to actions associated with semax, when L-NAME was introduced to adult animals there was a decrease of cognitive components in behavior reactions, probably due to its anxiogenic effects,.

The chapter contains experimental facts which were partly described earlier (Timoshenko et al., 2009 a, b) and novel data as well, illustrating the possible changes which could be induced in the developing brain and behavior and which could be causally related to an increase in the intensity of postnatal brain proliferative activity.

# Methods

## Experimental Animals

1) Rats from the Krushinsky-Molodkina (KM) strain were maintained in the local rat colony (Laboratory of Physiology and Genetics of Behavior, Biology Department,

Lomonossov Moscow State University). They were bred for audiogenic epilepsy proneness starting from 1948 on the basis of the Wistar outbred strain; they were bred by brother –sister matings from the end of 1980. They are characterized by the maximal pronennes to sound induced seizures.

2) Wistar outbred rat strain. The parents of Wistar rats used in the study were purchased from the Stolbovaya Animal Farm (Russian Academy of Medical Sciences).
3) The main part of behavior tests were performed with two mouse strains -101/HY and C3H/He. The 101/HY mouse strain (Lil'p et al., 2000, 2002; Poletaeva et al., 1992, 1996b) was used,possessingused, possessing several CNS anomalies. It was established that this strain contains the mutated locus *mut-1*, which determines the defects in the DNA-repair process, induced by chemical mutagenes (Lilp et al., 2000, 2002). This strain was reported to have several CNS anomalies, which could be not due to the *mut-1* locus, but to newly developed small mutation events affecting the CNS, and which are probably due to an abnormal *mut-1* gene allele (Boiarshinova et al., 2009). This locus was not mapped.
4) C3H/He strain mice were used as the normal controls.

Both strains were inbred, and the parents of animals used in this study were received from the Svetlye Gory Animal Farm (Russian Academy of Medical Sciences). The audiogenic epilepsy changes as a result of the neonatal semax treatment were analyzed in DBA/2J, CDF/Lac/Sto and C57BL/6J mice (see Results).

Water and standard rodent food (suppliers – Laboratorkorm and MEST, Russia) were *at lib*. All experimental manipulations were performed in accordance with the Directive 86 of EU.

The number of animals (rat and mouse pups), sacrificed to evaluate neurogenesis intensity, is indicated in tables 1-4. The number of animals used in the behavior investigations was indicated in the respective parts of the chapter.

### *Neonatal Injections*

Rat and mouse pups were injected daily with neuropeptide semax (50mg/kg) or NO-synthase inhibitor L-NAME (L-NG-nitroarginine methyl ester, 50 mg/kg) during different time periods of early postnatal life (see tables 1 and 4). Pups were injected subcutaneously at the nape area over 5 days at different age intervals (see table 1). Rats used for behavioral tests were injected daily at the age from 7 to 11 days old. Mice, used for behavioral testing, were injected over a span of five days, starting from postnatal day 2. A similar injection schedule was used for neonatal fluoxetine injections (dose 5mg/kg), using mouse pups of both strains. These animals were tested in the extrapolation task (see below), and their neurogenesis level was not investigated. During the period of injections, the weight of the pups was measured daily, and no differences between experimental (injections) and control groups were found. Control animals were left intact. The second control group, the animals which were injected with saline (in order to control the effects of neonatal pain stimulation), was used for behavior experiments. The new cells count (see below) in the dentate area of the control pain stimulation group reveal no differences between intact control animals and saline injected controls.

### *Immunohistochemical Techniques*

The stimulating influences of neonatal injections on cell proliferation in the rat's dentate area were visualized by immunohistochemical staining using antibodies of a *Ki-67* protein (anti-Ki67 Nova Castra diluted with PBS 1:300), expressing in the nuclei of dividing cells (Winking et al., 2004).

In order to reveal the cells, which started the neuronal differentiation, the immunohistochemical staining used antibodies to doublecortin (DCX). The expression of this protein is associated with microtubules in neuroblasts and differentiating neurons (Couillard-Despres et al., 2005). The neuronal precursors start to express DCX shortly after the start of the cell-division cycle, and decrease this expression in 2-3 weeks (in mature neurons). These features make the DCX immunostaining fit to mark the neurogenesis (Gleeson et al., 1999).

After a lethal urethane injection, the brains of rats and mice were fixed by means of a transcardial perfusion with saline (0.9% w/v NaCl) followed by 4% w/v paraformaldehyde in 0.1 M phosphate buffer saline (PBS) pH 7.4. After the perfusion, the brains were post-fixed in the perfusion solution overnight at 4°C. The next procedure included incubating brains in 20% w/v sucrose in 0.1 M PBS (24 hours at 4°C). 40 micrometers of coronal sections were cut using frozen microtomes/cryostats for free floating immunohistochemistry. The sections were then rinsed in 0.1 M PBS, three times for 5 minutes each, and then incubated in a primary antiserum at 4°C for 24 hours under constant gentle agitation. Following incubation in the primary antiserum, the sections were rinsed in PBS and incubated for 60 minutes in a Texas Red labeled donkey secondary antiserum (Jackson ImmunoResearch Laboratories, West Grove, PA): 1:50 diluted with PBS – 0.3% Triton X-100. The following primary antibodies were used: anti-Ki67, made in a rabbit (Nova Castra), diluted with PBS 1:300; anti-DCX, made in a goat (Santa Cruz), diluted with PBS 1:100; anti-BRDU made in a mouse (ICN), diluted with PBS 1:10. The sections were mounted on a glass slide cover-slipped with glycerol and analyzed by means of a fluorescent microscope (Olympus Camedia C-4000 ZOOM).

The cell counts were performed in 5 sections of the dorsal hippocampal area, starting from the section separated by 240 μm from the anterior edge of the dentate gyrus, thus the sections analyzed included the dorsal part of the dentate gyrus. The digital images of the dorsal dentate area in the fluorescent light were obtained, and the visual counts were created by using the Adobe Photoshop 6.0.

### *Behavioral Tests*

#### Rats. Audiogenic Epilepsy

The intensity of the audiogenic seizures (AS), in response to loud sounds (the bell, 100 dB), and startle reactions were scored in arbitrary units. A sound attenuating a plastic box was used. In cases of a muscular seizure development, the sound was stopped. If the animal displayed no reaction to the sound, or only a clonic run developed, the sound duration was 1.5 minutes. The arbitrary units for startle reactions were: "0" - no reaction; "1" - weak startle; "2" - wince of all body muscles; "3" - startle with a jump. For audiogenic seizures, they were: "0" - no reaction to sound; "1" – motor excitation stage (wild run, or clonic run); "2" – clonic seizure; "3" – clonic-tonic seizures; "4" - tonic seizures with an extension of all extremities (Poletaeva et al., 2011;Poletaeva et al., 2011).

The proneness to audiogenic seizures was tested in 1--month-old animals of KM and Wistar genotypes in intact groups and in animals after neonatal treatments.

## Mice. Audiogenic Epilepsy

The study of AS proneness in mice was performed in the same box as in the rat experiment. In cases when there were no reactions in response to the sound, the sound lasted for 20 seconds. The sound was stopped at the first signs of the mouse's motor excitation (clonic run). The arbitrary units of audiogenic fit intensity were the following: "0" - startle reaction only; "1" – clonic run stage, which was interrupted by the quiescence "pause"; "2" – clonic run stage without quiescence "pause"; "3" – motor clonic-tonic seizure; "4" –death as a result of a seizure fit during sound exposure. Mice were tested at the age of 1 month.

## Rats, Mice. Open Field Test

A round (diamaterdiameter 100cm) arena with 8 x 8 cm squares (for mice), or 16 x 16 cm (for rats), had a central zone (16 or 8 squares, respectively) and 25 holes in the floor (to check the number of hole-pokes). The animal was placed in the center of the arena and visually observed for 3 minutes. The number of squares crossed in the arena's center and periphery, rearings, hole-pokes, grooming episodes and defecation boluses, as well as the durations of grooming and freezing episodes,wereepisodes, were recorded using a semi-automated program with further PC program processing (program of M. Pleskacheva).

## Rats, Mice. The Elevated Plus-Maze (EPM) Test

A black, plastic maze was elevated at 72 cm from the floor. An animal was placed in the EPM for 3 minutes. Two different size EPMs were used for rats and mice respectively. The number of open arm entries, the total time spent there, as well as the number of "hanging" over open arms, the total number of rearings, grooming and freezing episodes, and defecation numbers were manually recorded.

## Mice. The Extrapolation Test

The plastic box for the extrapolation task (35.5 x 23.5 x 11.5 cm) had three openings in one of the box's wider walls at floor level (Figure 1, a, c). This wall was "inserted", or "pressed in*",* into the box (for 4 cm) and serves as the "screen" (see Figure 1). The mouse (which was food and water deprived for 16-18 hours) was introduced into the box and started to drink milk from the small cup, placed in front of the central opening (Figure 1, a). As soon as the animal began to drink, the food cup started to move slowly to the right or to the left and stopped in front of one of the side openings. In order to reach the food again (in front of one of the side openings) the animal had to make a 90° turn so that it could follow the food cup's movement and get the food.

The control food cup, initially not shown to the mouse, moved towards the opposite direction and stopped at a distance of 1 cm from the second side opening. This procedure served to balance the milk's odor from both openings (Figure 1).

Thus, the correct task's solution consisted of the mouse moving to the food cup, displaced to the side, and drinking milk from it. The movement towards and peeping into the "empty" side opening was scored as an incorrect solution.

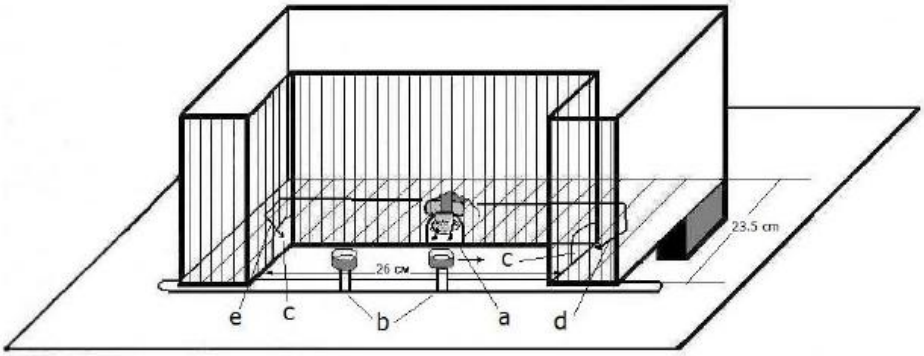



Figure 1. Extrapolation box used for experiments with mice (see text). a - the central opening, b – food bowls, c - side openings, d – the animal trajectory towards food (correct choice), e – the animal trajectory towards the "empty" side opening (incorrect choice). The schemer shows that the food moves to the right (arrow).

If the mouse, once being placed into the box, did not approach the central opening or did not drink milk from the cup, it was scored as having a "refusal" from the task's solutions; if the animal did not approach any side openings within 2 minutes of sliding the cup to the side opening then this reaction was scored as a "0" solution.

Each extrapolation task's experimental session included 6 trials; the direction of the food cup's movement changed from right to left in a "quasi-random" order (the food was not moved in the same direction for more than two times successively). The trajectory of the mouse's movement while searching for food, as well as the latency of finding the food, were manually recorded. The proportions of correct task solutions during the first task presentation, as well as for the whole number of trials, were used to measure the successfulness of the extrapolation task. The significance of the correct solution proportion difference from the 50% level, which is characteristic for a random performance, was evaluated.

### Statistics

The significance of differences in the labeled cell numbers in hippocampal dentate area between control and experimental brains (neonatal semax and L-NAME injections) were performed using a Student t-test or non-parametric criteria (Mann-Whitney, and median tests). The behavioral scores were compared using a two- or three-factorial ANOVA with a *post-hoc* LSD test. The differences between animal groups in the extrapolation test performance from the 50% chance level were evaluated by the φ method (Fisher criterion). A PC program, Statistica 6, was used.

## Results

There was neurogenesis in the subgranular zone of the hippocampal dentate area after the neonatal semax and L-NAME treatments in the rats. Practically all brain sections investigated contained cells with the nuclear marker Ki-67. The difference in the Ki-67+ cell numbers between control and neonatally treated rats are shown in Figure 2.

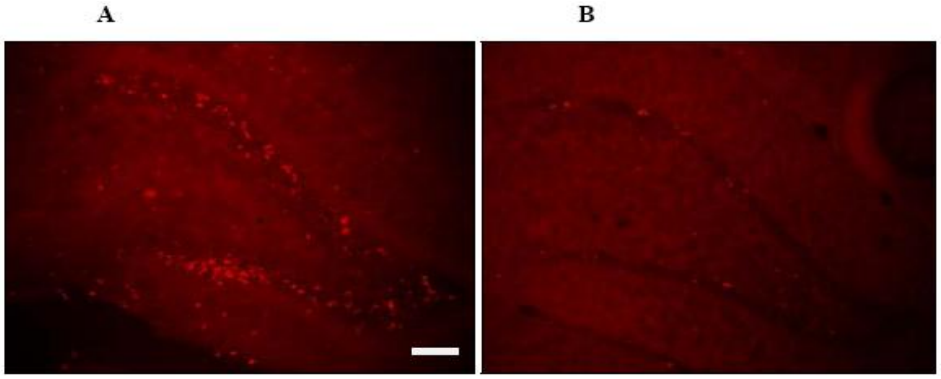



Figure 2. The sections of the hippocampal dentate area in a KM rat's brain. Immunohistochemical staining (see Methods) for Ki-67. A – 14-day-old rat, daily semax injections on days 7-11, B – the brain of an intact rat of the same age. Bar – 100 μm.

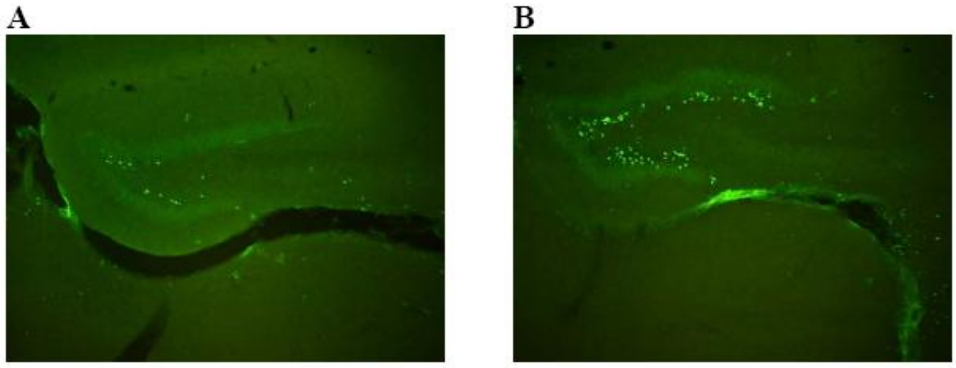



Figure 3. Microphotographs of the dentate area section, immunohistochemical staining for BrdU. A- the brain of a 20-day-old mouse of the C3H/He strain after neonatal semax injections, B - brain of an intact mouse of the same age and strain. Scale bar - 100 μm.

Table 1 presents data on the mean cell numbers that were marked by the antibody *Ki-67*. With one exception (Wistar rats perfused on the $17^{th}$ postnatal day after a semax injected during the $10^{th}$ -$14^{th}$ days), the cells that were immunopositive to *Ki-67* were significantly more numerous after semax injections (Mann-Whitney, $p<0.05$). In cases when the brain fixation had been performed on the day following the treatment, the difference between experimental and control groups were maximal, although in cases of a 9-day interval between the injections and brain fixation, the difference still had been significant. The postnatal neurogenesis at the ages investigated was rather intense in the control brain too, as the peak of proliferation intensity occuredoccurred at this age (Faiz et al., 2005), but the semax and L-NAME (see below) injections induced a further increase in the number of dividing cells.

The absolute cell numbers in the experimental series after neonatal L-NAME injections (NOS inhibitor, which would presumably increase the intensity of postnatal neurogenesis) were lower than in the semax series, although the direction of changes, as aresulta result of treatment, was the same as in the case of semax. Thus, daily injections of L-NAME to rat pups in the early postnatal ages induced an increase in the number of new cells in the dentate area of a hippocampal formation (Table 2).

**Table 1. Mean numbers of *Ki-67* immunopositive cells in a rat's subgranular zone of the hippocampal dentate area in both hemispheres (5 sections per brain) in control animals and after the semax neonatal injections (Wistar and KM strain)**

| Rat strain | injections age | perfusion | n | semax | control |
|---|---|---|---|---|---|
| KM | $7^{th}$ to $11^{th}$ postnatal days | $13^{th}$ day | semax-2, control -2 | 280,43±7,9 * | 195,13±7, 9 |
| | $13^{th}$ to $17^{th}$ postnatal days | $18^{th}$day | semax-3, control -3 | 106,73±3,2 * | 58,93±3 |
| | $10^{th}$ to $14^{th}$ postnatal days | $24^{th}$day | semax-4, control -4 | 99,62±7,7* | 58,6±2,1 |
| Wistar | $10^{th}$ to $14^{th}$ postnatal days | $17^{th}$ day | semax-5, control -4 | 146,37±7,9 * | 94,77±3,8 |
| | $10^{th}$ to $18^{th}$ postnatal days | $25^{th}$day | semax-4, control -4 | 71,7±4,8* | 35,75±4,6 |
| | $10^{th}$ to $14^{th}$ postnatal days | $17^{th}$ day | semax-3, control -3 | 89,5±3,2 | 51,4±6,9 |

n - number of animals, perfusion – the age of brain perfusion. * - significantly different (Mann-Whitney) from the number of cells in the respective control group, $p< 0.05$.

**Table 2. Numbers of *Ki-67* immunopositive cells in rats in a subgranular zone of the hippocampal dentate area of both hemispheres (5 sections per brain) in control animals and after the L-NAME neonatal injections (Wistar and KM strain)**

| Rat strain | injections age | Perfusion age | n | L-NAME | Control |
|---|---|---|---|---|---|
| KM | 7th to 11th postnatal days | 15th day | L-NAME-3, control - 3 | 61,2±3,4* | 30,4±7,9 |
| Wistar | 9th to 13th postnatal days | 18th day | L-NAME-4, control - 4 | 60,3±2,7* | 44,7±2,4 |

* - significantly different (Mann-Whitney) from the number of cells in the respective control group, $p< 0,05$, n- number of animals.

It was demonstrated that both treatments (the daily injections of neuropeptide semax and of NOS inhibitor L-NAME), during the first two weeks of postnatal life in rats of two genotypes, induced a significant increase in the number of newborn cells, be revealed not only during the course of injections, but also after they were finished. The effects of the semax injections (firstly demonstrated in these experiments) were more clear-cut. No significant genotype differences in the scope of these effects were found.

The next goal was to determine whether the population of neuronal precursors of the dentate area was also affected by the treatments used. The immunohistochemical staining for doublecortin (see Methods) demonstrated (table 3) that the neonatal semax increased the neuroblast numbers in the hippocampal dentate area (neonatal semax - 36+2,8, control - 21,8+1,2). The DCX immunostaining had not been performed in the L-NAME injected rats group. It should be noted that the number of DCX-immunopositive cells (neuroblasts and young neurons) was much more numerous in Wistar brains, in comparison to those of the KM strain, especially after semax treatments (table 3).

**Table 3. The mean numbers of *DCX*-immunopositive cells in the subgranular zone of the dentate area of hippocampal formation in control group and after semax injections in KM and Wistar rats (5 sections per brain)**

| Rat strain | injections age | perfusion | *n* | Semax | control |
|---|---|---|---|---|---|
| KM | 10th - 14th postnatal days | 23$^{d}$ day | semax-4, control -4 | 93,5±14,2$^{*}$ | 50,3±5,2 |
| Wistar | 10th - 14th postnatal days | 17$^{th}$ day | semax-5, control -5 | 208,8±8,4$^{**}$ | 90,9±9,3 |

The differences (Mann-Whitney test) between KM and Wistar are significant (p=0,02 for control groups, p=0.037), *, **- significantly different from the control group scores p<0.05 and p<0.01, respectively.

**Table 4. The mean numbers of *Ki-67* immunopositive cells in a mouse's subgranular zone of the dentate area (strains 101/HY and C3H/He) after semax neonatal injections and in the control**

| Mouse strain | Injection age | Perfusion | n | semax | control |
|---|---|---|---|---|---|
| 101/HY | 5 - 10 postnatal days | 11$^{th}$ day | semax - 4, control-4 | 153,5±13,6* | 109,3±2,7 |
| | 7 - 11 postnatal days | 22th day | semax - 3, control-3 | 35,8±1* | 26,2±2,1 |
| | 7 - 11 postnatal days | 14th day | semax - 3, control–3 | 53,2±0,5* | 38,8±2,8 |
| | 11 - 15 postnatal days | 24th day | semax - 6, control–6 | 30,1±2**$^{*}$ | 11±1,3 |
| C3H | 5 - 11 postnatal days | 11th day | semax - 3, control-3 | 135,1±5,4* | 116,6±1,6 |
| | 7 - 11 postnatal days | 20th day | semax - 3, control-3 | 47,7±1,6$^{*}$ | 32±2,9 |
| | 16 - 20 postnatal days | 23th day | semax - 3, control–3 | 50±0,2* | 35,8±1 |
| | 10 - 14 postnatal days | 23th day | semax - 3, control–3 | 31,3±2,5** | 22,9±0,9 |

n- number of animals, * - significantly different (Mann-Whitney) from the cell number values in the respective control group, p< 0,05.

The number of cells immunoreactive to *Ki-67* was larger in those cases when brains were immediately perfused after the injections than in cases when the perfusion was performed at an interval of several days after the end of the injections.

*Mice.* Experiments with neonatal semax and L-NAME injections ono mouse pups of 101/HY and C3H genotypes also demonstrated the stimulating effects of these treatments on SGZ proliferation activity. The significant increase of *Ki-67*-labelled cells in mouse brains after neonatal semax injections can be seen in table 4.

The immunohistochemical staining for DCX (neuronal precursors visualizing) was performed in the brain sections of C3H mice only. As in the rat experiments, a significant (p<0.01) increase in neuronal precursors took place in the mouse brain after neonatal semax.

In total, the increase of proliferating cell numbers in the mouse brain was not as large as in the rat's. This difference could be attributed to interspecies differences.

A small experimental series, in which proliferating cells were marked by means of specific antiBrdU staining, demonstrated that neonatal L-NAME injections were also followed by an increase in numbers of BrdU-immunopositive cells in the SGZ (41,25+20,8 – for L-NAME treated mice and 34,3+15,8), although the stimulating effect of L-NAME did not appearintenseappear intense. It is possible to suggest that these differences from the data with *Ki-67* immunoreactive cells staining are due to BrdU metabolism peculiarities (Bagley et al., 2007).

Data obtained showed that neonatal semax and L-NAME treatments increased the proliferation activity in the hippocampal dentate area in the brains of rats and mice. The latter means that this effect could be one of the factors which determine the changes in several behavioral indices revealed in these animals later on in life, at the ages of 1 month (audiogenic epilepsy predisposition) and 2 months old (open field, elevated plus-maze and extrapolation tests, see below).

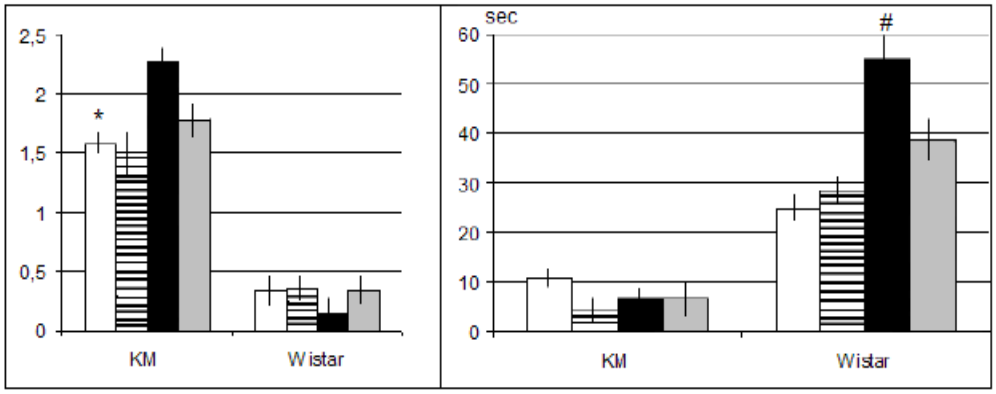
## *Behavior. Experiments with Rats*

### Audiogenic Seizures in KM and Wistar Rats after Neonatal Treatments

Males and females of the KM (audiogenic seizure prone) and Wistar strains (with low audiogenic seizure proneness) were used in this series. At the age of 7 to 11 postnatal days, rat pups were injected daily with 50 mg/kg L-NAME (32 KM and 14 Wistar rats) or 50 mg/kg semax (9 KM and 12 Wistar rats); 39 KM and 13 Wistar rats were used as intact controls. Audiogenic epilepsy proneness was tested at the age of 1 month. This age at testing provided the possibility to observe both plausible changes in audiogenic fit intensity – the increase or the decrease of this trait expression. In adult rats of the KM strain, the seizure fit of maximal intensity developed at the age of 3 months, whereas seizure proneness is lower in younger animals. Nonetheless, about 100% of KM rats display the trait (Poletaeva et al., 2011; Poletaeva et al., 2011).

A three-factor ANOVA (strain, sex, treatment) revealed no significant effects of sex on AS parameters in KM and Wistar rats. For further analysis, pooled data was used on the AS scores for males and females. A two-way ANOVA for audiogenic fit intensity (in arbitrary scores) demonstrated a significant effect of the "strain" factor ($F_{1\text{-}96}$=74.585, $p$=0.0000) and a tendency for an interaction between the "strain" and "treatment" factors ($F_{1\text{-}96}$=2.578, $p$=0.0804). An LSD analysis demonstrated that AS intensity was significantly ($p$=0.0008) higher in the KM rats' group after early semax administration in comparison to the intact control group (2.16±0.15 and 1.46±0.13, respectively). The neonatal L-NAME injections in KM rats increased the audiogenic fit intensity (non-significantly).

Figure 4 shows the effects of semax and L-NAME neonatal injections; these treatments changed the AS scores in KM and Wistar rats. A nonparametric median test revealed significant effects associated with the neonatal treatments in KM rats ($\chi^2$=15.27, df = 2, p=0.0005). At the same time, changes in the AS fit intensity in Wistar rats after neonatal exposures were small and insignificant. The mean audiogenic fit intensity in Wistars was significantly ($p$=0.0000) lower than in KM rats (in intact control groups 0.31±0.23 and 1.46±0.13, respectively, arbitrary units).




a b

Figure 4. Audiogenic epilepsy scores in 1-month-old rats of two genotypes after neonatal semax and L-NAME injections. Bars: white – intact animals, stripped – neonatal pain stimulation (saline neonatal injections), black – neonatal semax, grey –neonatal L-NAME. A – mean audiogenic seizure fit intensity (see Methods), ordinate - arbitrary units of AS. B – mean fit latency (ordinate – sec). * - significantly different from the neonatal semax group, $p<0.05$ (Mann-Whitney), # - significantly different from the intact group, from neonatal pain stimulation and neonatal L-NAME groups, $p<0.01$.

The analysis of fit latencies gave the following results: the two-way ANOVA revealed significant effects of both factors ("strain" - $F_{1\text{-}96}=76.01$, $p=0.0000$, and "treatment" - $F_{1\text{-}96}=18.74$, $p=0.0000$). There was only a tendency to decrease the fit latency in KM rats (LSD test). As the number of animals which developed seizures in Wistar group was not large, the respective data was analyzed by means of a nonparametric median test. It showed a significantly ($\chi^2= 6{,}00$, df = 2, p =0.05) longer latency in the semax neonatal injections group (see Figure 2).

At the same time, the neonatal injections affected more than fit intensity and fit latencies in 1-month-old rats. The sound exposure was the cause of the death for several animals during a period up to 12 hours after the sound exposure: 3 out of 9 KM neonatal semax injectees died after sound exposure, while no deaths occurred among the KM rats, which received neonatal L-NAME, and among Wistars after both treatments and control animals of both strains.

Thus, interstrain differences were revealed in the remote effects of early (neonatal) semax and L-NAME administration, discovered in audiogenic seizure parameters.

The remote effects in audiogenic epilepsy as the result of early treatments were also noted as changes in the audiogenic seizure pattern. In rats of both genotypes and after both types of treatments, the seizure developed in the aberrant way. Animals displayed clonic seizures with an unusual posture, "on the back", which had been observed neither in intact rats nor after the administration of any pharmacological agents. Another unusual reaction to loud sound in these rats had been the quick development of a cataleptic-like state during a seizure fit (and not after the cessation of seizures, as is the norm). Such a phenomenon also never occurred in normal rats. In these cases, the animals stopped, frozen, and kept the unnatural posture with muscle contractions being maintained.

The intensity of acoustic startle reactions was also altered as a result of early semax and L-NAME administration: a two-way ANOVA revealed a significant effect of the "strain" factor ($F=11.30$, $p=0.001$), of the "treatment" factor ($F=9.19$, $p=0.0002$), and a significant interaction of them ($F=5.40$, $p=0.0058$).

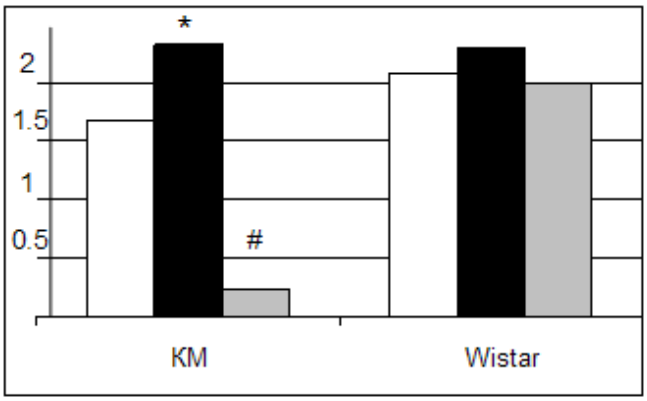



Figure 5. Startle reaction in KM and Wistar rats in response to the sound onset. Ordinate – the startle intensity in arbitrary units (see Methods). Bars - as in figure 4. * - significantly different from the intact group, $p<0.05$, # - significantly different from the neonatal semax group, $p<0.01$.

In Wistar rats the startle was more intense and was practically unaltered by early treatments (2.08± 0.28, 2.28±0.27, 2.00±0.29). At the same time, neonatal semax injections in KM rats significantly enhanced startle intensity (in comparison to the intact group, $p$=0.0089), whereas neonatal L-NAME inhibited the startle response.

It was previously shown, that the period of NOS activity inhibition after L-NAME administration was rather short (Bashkatova et al., 2001), and its duration was shorter than the time between the end of L-NAME injections in our experiments and audiogenic seizure testing. The same was true for semax, although no direct evidence exists. Thus, NOS inhibition (after L-NAME administration) did not take place (already for a long time) at the moment of audiogenic seizure testing. The latter means that a plausible explanation for changes in audiogenic epilepsy proneness in our experiments would be associated with the remote effects of neonatal treatments, presumably involving neurogenesis modulation.

## Open Field Test. Locomotion

No sex differences were revealed by the three-factor ANOVA (genotype, sex, treatment) for squares crossed in the arena's periphery. A two-factor ANOVA (genotype, treatment), for the 1st minute of the test, revealed a significant genotype effect ($F_{1\text{-}96}$=24,9759, p=0,0000); Wistar rats demonstrated higher locomotion in both intact- and semax-treated groups, in comparison to the respective KM groups (LSD *post hoc* p=0.0008, and p=0.0456) (Figure 6). The summarized periphery locomotion scores, after 3 minutes of testing (LSD *post hoc)*, were higher in Wistar rats after semax neonatal injections ($p<0.05$) than in the controls. No sex differences were revealed in the three-factorANOVA, but they were revealed by a non-parametric median test. Higher scores of peripheral locomotion were found in neonatal semax females ($\chi^2$= 8,618182, df = 2, p = 0,0134) in comparison to control females, while no differences were found in males. For the second minute of open field peripheral locomotion, two-factor ANOVA revealed a significant effect for both "strain" ($F_{1\text{-}96}$=7.16, p=0.009) and "treatment" ($F_{1\text{-}96}$=3.26, p=0.045) factors, as well as for their interaction ($F_{1\text{-}96}$=3.27, p=0.0441). The treatment effects in scores of KM rats for third minute locomotion were not significant.

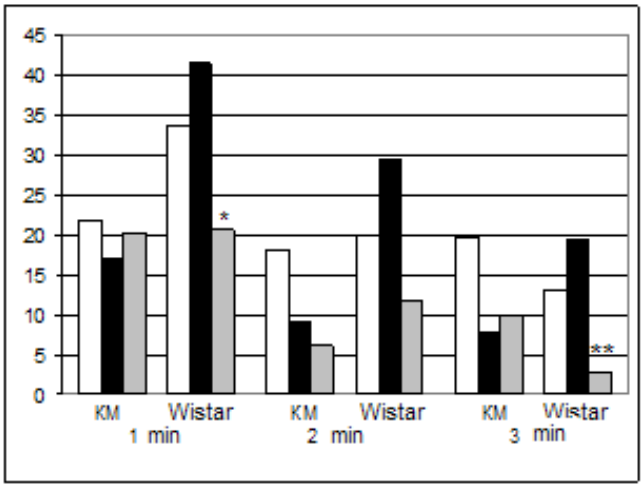



Figure 6. Open field test, rat experiment. The locomotion scores in the arena's periphery (ordinate - number of squares crossed) for 3 minutes of the test, presented separately. Bars as in figure 4. * - significantly ($p<0.05$) different from intact rats, ** - significantly ($p<0.01$) different from intact and neonatal semax groups.

The *post hoc* LSD for KM rats of groups investigated showed no significant differences, while Wistar neonatal L-NAME group animals were significantly less active than the semax group and their controls.

Figure 6 shows that locomotion scores of intact KM rats did not change during the three minutes of testing (demonstrating no habituation in these animals), while the effect of habituation was seen in intact Wistars. The habituation was also obviously present in all groups of neonatal treatments, especially in Wistar L-NAME animals.

It is possible to suggest that interstrain and intergroup differences in locomotor activity indices were determined by differences in reactions to a novelty (animal being placed into an open field arena), while the decline of open field locomotion in the arena's periphery demonstrated a habituation process. This pattern was not seen in KM rats, but was quite efficient in Wistars.

The number of squares crossed in the center of the open field arena (Figure 7) is the "reverse" indicator of animal fear (anxiety). The more frequently an animal visits the center of the arena and the longer the time it stays there, the less its behavior is influenced by a fear-anxiety state. In intact Wistar and KM rats, the scores of this variable for a 3-minute interval were more or less equal and not high (animals rarely visited the arena's center). The three-factor ANOVA showed a significant effect associated with sex on this score for the 1st minute of the test ($F_{1\text{-}90}=5.38$, $p=0.0226$): The LSD *post hoc* showed that Wistar females visited the arena more frequently. However, in the KM strain, the effect of the "sex" factor was insignificant.

The two-factor ANOVA demonstrated significant effects of both "strain" ($F_{1\text{-}96}=17.68$, $p=0.0000$) and "treatment" ($F_{1\text{-}96}=5.54$, $p=0.0053$) factors. The interaction of both factors was also significant ($F_{1\text{-}96}=8.55$, $p=0.0004$), reflecting the fact that there were practically no significant differences between intact and treatment groups in KM rats as well no differences between KM and Wistar intact groups (the intergroup variability inside KM groups was very high).

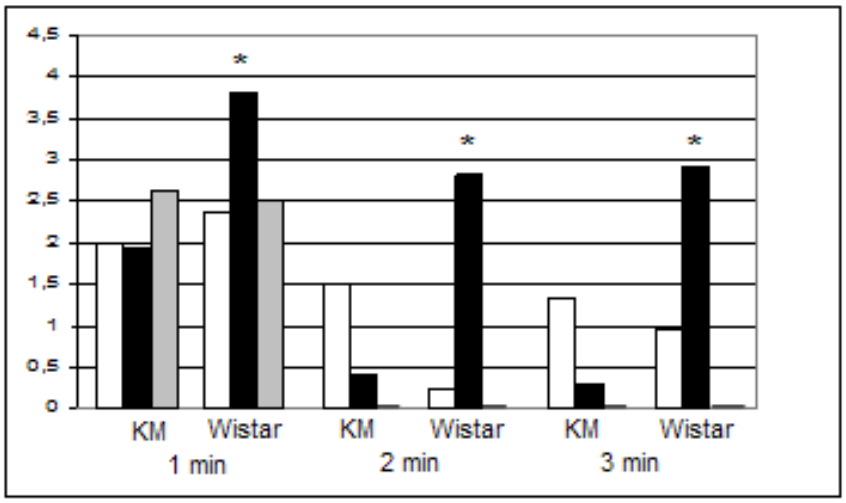



Figure 7. Open field test, rat experiment. The locomotion scores in the center of the arena for 3 minutes of the test. Ordinate - number of squares crossed. Bars - as in Figure 4. * - significantly ($p<0.001$) different from scores of intact animals and of rats after neonatal L-NAME.

The locomotion in the center of the arena was consistently higher in the Wistar neonatal semax group throughout the entire duration of the test ($p=0.0000$), whereas a neonatal L-NAME injection was followed by the abrupt decrease of "central squares" in both strains during the second and third minutes of the test. This fact could be due to the increase of fear-anxiety in rats after neonatal L-NAME.

Thus, in rats of the Wistar strain, the fear-anxiety state which developed in response to placing the animals into a novel open space was much lower after neonatal semax (than in intact animals) and was higher (than in intact rats) after neonatal L-NAME. The latter effect was also seen in KM rats. This means that two types of neonatal treatments (semax and L-NAME) were accompanied by opposite changes in anxiety levels.

The preliminary conclusion was that neonatal injections of semax and L-NAME influenced both open field locomotion variables - squares crossed in the center and at the periphery. This effect was revealed in 2-month-old animals, i.e. in 7 weeks after the end of injections. It is possible to state that these changes represent the remote effect of the neonatal treatment used.

## Open Field. Exploratory Behavior

Rodents placed in the open field environment reveal species-specific exploratory behavior, rearings (upright postures) and hole-poke movements being among them. The hole-poke numbers showed no differences between the experimental groups investigated whereas differences in rearing numbers were numerous (Figure 8).

A three-factor ANOVA (sex, strain and treatment) revealed a significant effect associated with "strain" ($F_{1\text{-}90}=10.90$, $p=0.0014$) and "sex" ($F_{1\text{-}90}=5.38$, $p=0,0226$) factors, as well as a significant interaction of "strain" and "treatment", and the interaction of all three factors ($F_{1\text{-}90}=5.90$, $p=0.0040$). This means that the number of vertical postures significantly differed in both directions of the two strains and in treatment groups. The two-factor ANOVA revealed a significant interaction between "strain" and "treatment" ($F_{1\text{-}96}=7.07$, $p=0.0014$).

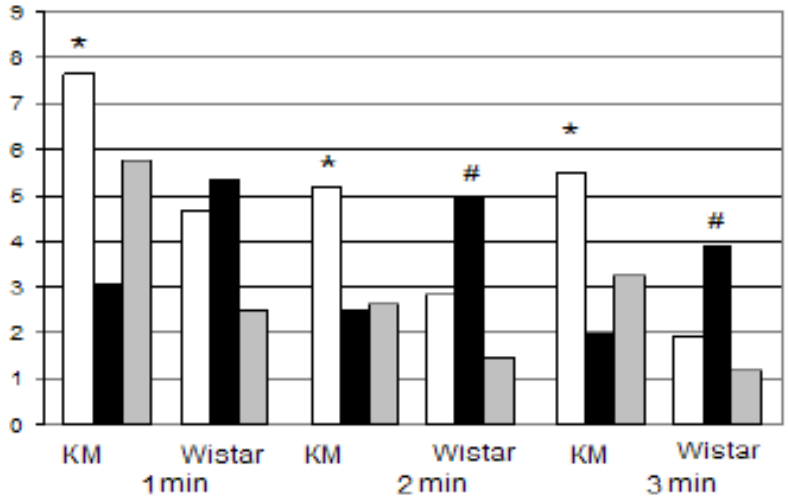



Figure 8. Open field test. Numbers of vertical upright postures (rearings) in successive test minutes. Ordinate – number of rearings. Bars - as in Figure 4. * - significantly different ($p<0.05$) from neonatal semax group, # - significantly ($p<0.05$) different from intact and neonatal L-NAME groups.

For all three minutes of the test, the number of rearings had been higher ($p=0.025$) in KM intact rats in comparison to the same Wistar group (12,00±1.65 and 7.53±1.06, respectively).

The number of rearings in the KM neonatal semax group was significantly ($p=0.0434$) lower than in intact KM ratsFurthermorerats Furthermore, the difference was also significant in Wistar rats ($p=0.0022$), but was opposite in sign – rearings were more numerous in the neonatal semax group than in intact animals. Neonatal L-NAME groups of both genotypes were not different from the intact animals. As the number of rearings in the open field is usually negatively related to fear-anxiety indices, the data confirms our previous conclusion that the Wistar neonatal semax group demonstrated a decrease in anxiety after this neonatal treatment. At the same time, this inverse relationship was not maintained by the case of neonatal L-NAME. The increase of fear-anxiety in rats of both genotypes (the decrease of central squares crossed) after neonatal L-NAME was in contrast to the substantial numbers of rearings in these groups. As upright postures were mainly performed in the open field's periphery, these facts are mutually contradictory: the increase of the fear state could not be accompanied by the increase of exploratory movements according to a traditional understanding associated with the meaning of these indices.

## Open Field. Grooming

The number of grooming episodes and their total durations were estimated during open-field exposure. The fur cleaning behavior (grooming) is the innate behavioral element which is important, with respect to hygenics. Grooming behavior is an important index of a rodent's open field behavior. Moreover, grooming episodes in rodents occur more frequently in an environment which provokes fear and anxiety in them. It is more or less accepted that grooming scores in a novel environment will reflect a state of conflict between explorative- and fear-motivated states. No sex and genotype effects were revealed in the three-factorial ANOVA: the effect of the "treatment" factor was the only one with a significant effect ($F_{1-90}=9.95$, $p=0.0001$), although the factors' interaction was also significant ($F_{1-90}=3.54$, $p=0.0331$- for treatment-genotype, and $F_{1-90}=5.14$, $p=0.0077$ for all three factors), meaning

that changes in treatment groups in the two rat strains were of opposite directions. In particular, differences in all female groups were insignificant. All KM males displayed more grooming episodes (intact - 7.89±1.08, neonatal semax - 11.00±1.15, neonatal - L-NAME 11.00±1.87) in comparison to Wistar males (6.40±1.45, 2.55±0.76, 3.86±1.23, respectively). The tendency (p=0.0548) towards grooming increases after neonatal semax was noted in KMs, whereas the number of grooming episodes significantly decreased in Wistars (p=0.0237). Keep in mind that the decrease in fear-anxiety (by the "central squares" index) and increase in rearings took place in the Wistar neonatal semax group. Changes of the same direction were characteristic for grooming time and for both scores for three minutes in total.

## Open Field. Freezing

Freezing is a species-specific fear reaction that appears in other animals than rodents. The three-factorial ANOVA showed a significant effect associated with the "treatment" factor (F=9.95, p=0.0001), probably due to the fact that this reaction was not numerous in these experiments. The number of freezing episodes in females was not influenced by "strain" and "treatment"; the freezing score was maximal after neonatal L-NAME (p=0.0000) in KM males in comparison to other groups. This corresponded with the decrease of central squares crossed (increased fear-anxiety). A similar pattern of differences was obtained for the freezing duration scores.

## Elevated Plus-Maze (EPM)

A two-factor ANOVA showed a significant effect of the "treatment" factor ($F_{1\text{-}90}$=6.98, p=0.0015) for the number of "hangings over" (exploration index), the maximal number of these behavioral acts being in both intact groups. These scores were lower in groups with neonatal treatments, and the difference was more clear- cut in the KM strain (p=0.0075 for semax group in comparison to intact rats, and p=0.0341 – for L-NAME group). The neonatal L-NAME group was the only one different from intact (p=0.0199) Wistar rats. The time spent in the open arms of the EPM (anxiety index) was significantly lower in groups after neonatal treatments (2-factorial ANOVA - $F_{1\text{-}90}$=7.47, p=0.0010 for "treatment" factor). This time was maximal in the intact group and higher in KM rats (p=0.0164). Neonatal treatments drastically reduced these scores in both genotypes with more pronounced differences in KM groups (p=0.0049 for neonatal semax and p=0.0008 for L-NAME).

In summary, the 2-month-old KM and Wistar rats demonstrated behavior changes as a result of the neonatal semax and L-NAME administration. The avoidance of an open, brightly lit space (open EPM arms), which is characteristic for rodent anxiety behavior, significantly increased in rats of both genotypes as the result of both treatments. However, this was stronger in the KM strain. The reduction of explorative "hanging over" reactions was, of course, the trivial result, as rats spent less time in the open arms.

Thus, open field and EPM tests performed on rats of two genotypes revealed two effects. –First werethewere the remote effects demonstrated from neonatal treatments (semax and L-NAME administration).–Second, was that the "sign" and the scope of these effects were different in rats of two genotypes. Together with genotype differences in audiogenic seizure proneness (see above), the data on behavior modulation by neonatal semax and L-NAME could be qualified as the differential remote effects of early drug administration.

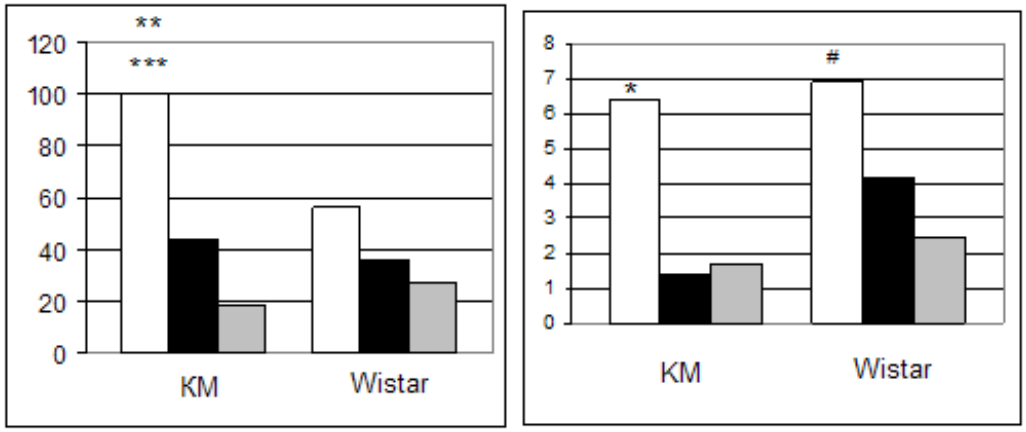



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Figure 9. Elevated plus-maze test. Neonatal treatment influence for A - mean time in the open EPM arms, and for B – number of "hanging over" reactions. Bars – as in Figure 4. # - significantly ($p<0.05$) different from neonatal L-NAME group. ** - significantly ($p<0.05$) different from neonatal semax group ($p<0.01$), *** - significantly ($p<0.05$) different from neonatal L-NAME group ($p<0.01$).

## *Behavior. Experiments with Mice*

### Audiogenic Seizure Susceptibility

Audiogenic seizure proneness was investigated in male and female mice of 5 strains after neonatal semax treatments. Audiogenic seizures were investigated in 220 mice in total (DBA/2J, n=42, CBA/Lac/Sto, n=38, C3H/He, n=45, 101/HY, n= 62, and C57BL/6J, n=33). Mice were tested at the age of 1 month, and arbitrary scores were used for an audiogenic seizure fit evaluation (see Methods).

There were no sex differences in the scores of the mice's audiogenic seizure fits (three-factorial ANOVA), whereas a two-factor ANOVA showed a significant effect of the "strain" factor ($F_{1,4} = 32.33$, $p=0.0000$) and a tendency towards significance for the "treatment" factor ($F_{1\text{-}2}=2.92$, $p=0.056$), as well as a significant effect associated with their interaction ($F_{1,8}= 6.86$, $p=0.0000$), due to opposite changes in semax-treated groups in CBA and DBA mice. The data for the audiogenic seizure proneness for mouse groups tested is presented in table 5.

**Table 5. The mean values (± error ) of audiogenic fit intensity (in arbitrary units) for 15 groups of 5 mouse strains (age of 1 month)**

| Mouse strain | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | semax | | saline | | intact | |
| | n | fit intensity | n | fit intensity | n | fit intensity |
| DBA/2 | 19 | 1,16***#±0,23 | 11 | 4,00***±,30 | 12 | 2,00±0,29 |
| CBA | 6 | 1,21±0,18 | 7 | 0,08±0,26 | 28 | 0,84±0,24 |
| 101/HY | 29 | 1,21±0,19 | 18 | 0.96±0,18 | 15 | 0.95±0,19 |
| C3H | 15 | 0.85±0.25 | 16 | 0,08±0,26 | 14 | 1,21±0,26 |
| C57BL/6 | 12 | 0,08±0,26 | 14 | 0,00±0,27 | 7 | 0,00±0,38 |

n – number of animals, * ** *** significantly different from intact group scores, $p<0.05$, 0.01 and 0.001, respectively, # - significantly different from saline group scores, $p<0.001$.

In spite of the fact that 101/HY and DBA/2J, in contrast to three other strains, were known to be audiogenic seizure prone (Newmann, Collins, 1991; Poletaeva et al., 1996), the neonatal semax and pain stimulation (saline group) changed this trait in DBA/2J, but not in 101/HY (the seizure fit parameters were almost same in all three groups). The neonatal semax increased (at the level of the tendency) audiogenic seizure pronenessin CBA mice, a change which was opposite to an effect of the same treatment in DBA mice (as mentioned above, this was revealed by a significant effect of the two factors interacting).

In spite of the fact that the sound had always been switched off as soon as the wild run stage started, the sound exposure still caused death in some of the animals. The DBA/2J mice dominated among the animals which died during the course of the seizure. There were 21 DBA/2J mice among them and only 6 mice of all other genotypes. The prevalence of DBA/2J mice among the animals that died could be explained by the fact that the sound exposure was performed at the age of DBA/2J's maximal audiogenic fit proneness (Newman, Collins, 1991). From the DBA/2J dead mice, 11 animals (out of 11 ) belonged to the saline group, 7 mice were intact (58 %) and only 3 were from the neonatal semax group (16%). Thus, this significant ($p<0.01$ and $p<0.001$, method φ) difference in the proportion of mice dying as the result of a seizure reflects the neonatal semax protective effect on DBA mice. The high mortality incidence in the DBA/2J saline group was in accordance with the fact that neonatal saline administration induced a significant (and genotype dependent) increase in pain sensitivity in adult rats and mice (Alexeev et al., 2003; Boyarshinova et al., 2004 b; Salonin et al., 2004; Beggs et al., 2011). It is possible to suggest that the protective effect of the neonatal semax treatment, clear-cut in DBA/2J mice, was probably not revealed in 101/HY mice due to differences in brain catecholamine levels as demonstrated earlier (Boyarshinova et al., 2004 a). As the neurogenesis in the DBA/2J strain was not analyzed in our work, one of the arguments regarding a current explanation of this data is the fact that DBA/2J mice revealed a less intense neurogenesis than did animals of the C3H strain (Kempermann, Gage, 2002 a, b). The vulnerability of the developing brain to treatments affecting brain excitability was demonstrated earlier. The neonatal anti-epileptic drug administration affected postnatal brain development in rats (Lombardo et al., 2005), and neonatal caffeine and pyracetame also induced changes in the audiogenic epilepsy proneness in mice of three genotypes (Markina et al., 2006).

## Open Field Test

Data on the remote effects of semax and L-NAME neonatal administration were obtained in different seasons. The open field behavior scores were, to a large extent, similar to those for the semax group and were not presented, as the group's size was too small for neonatal L-NAME. Thus, only neonatal semax treatment data for open-field and EPM tests are presented in this chapter. In the experiments with neonatal semax, 2-month-old male and female mice of the 101/HY (31 intact, n=31, 20 saline, saline n=20, semax, n-43) and C3H strains (intact, n=12, saline, n=28, semax, n=38) were used.

The locomotion level in the arena's peripheral zone was higher in the C3H/He strain than in the101/HY strain ($p<0.01$), while neonatal pain stimulation decreased this score in comparison to intact animals, and neonatal semax increased it, thus exerting some type of "normalizing" effect (Figure 10), as was in case of the DBA mice's death proportion in the audiogenic seizures experiment.

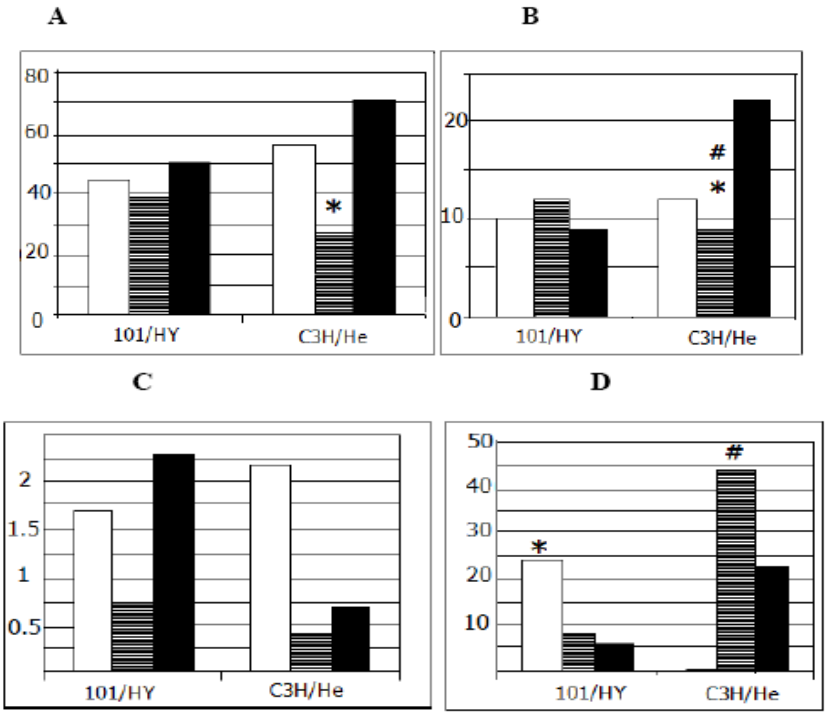


Figure 10. Open field test, mouse experiments. Remote effect of neonatal semax and pain stimulation (saline injections). A, B – numbers of squares crossed in the arena's periphery and center during 3 minutes of the test, respectively. Ordinate – number of squares. C - number of grooming episodes (ordinate), D - number of vertical postures (rearings, ordinate). Bars - as in Figure 4. * - significantly ($p<0.05$) different from the neonatal semax group, # - significantly ($p<0.05$) different from the intact group.

Figure 10 demonstrates that the number of squares crossed in the center was almost equal in intact animals and changed in the treatment groups in C3H/He mice, but not in 101/HY mice. Thus, semax injections reduced anxiety levels in C3H/He mice (increasing the number of central squares visited). These experiments demonstrated that the neonatal pain stimulation resulted in an obvious remote effect, changing the open field behavioral score. This data confirmed the earlier obtained data from our group which also demonstrated the "normalizing" effect of neonatal semax when changes were induced as the remote effect of neonatal saline administration. The interstrain differences in the open field behavior scores, presented in this chapter, also reveals a significant effect associated with factor interactions. The factor interactions reflect the fact that the scores of two genotypes were changed in opposite directions as the result of treatment. This effect could be seen in the scores for squares crossed in the center ($F=10.18$, $p= 0.0000$). The number of grooming episodes increased in the 101/HY neonatal semax group, but did not change in comparison to the saline group in C3Hs. The number of defecating boli was higher in 101/HYs (in all groups) in comparison to C3H scores and did not change as the result of neonatal treatments in either strain. Thus, the defecation rate, as the index of emotional reactivity, revealed no remote effects to neonatal treatments. The latter is one more fact which should alert researchers to cautiously consider this physiological variable as being causally related to fear-anxiety expression.

## Elevated Plus-Maze (EPM) Test

Male and female mice of both genotypes were used in this experiment: 101/HY (intact, n=17, neonatal saline , n=25, neonatal semax, n=19); and C3H/He (intact, n=4, neonatal saline, n=10, neonatal semax, n=15). No significant sex differences were found. The two-factor ANOVA demonstrated a significant effect of the "treatment" factor (F=4.41, p=0.038) on the number of open arm entries, the informative index of animal anxiety. The neonatal pain stimulation induced the strongest effect (the data for C3H/He groups were not significant due to the small size of the intact group) (Figure 11).

The remote effects of neonatal semax were obvious with the decrease in anxiety. The number of open arm entries and time spent there were higher in semax treated 101/HY mice, whereas this neonatal treatment was not effective in the C3H/He strain.

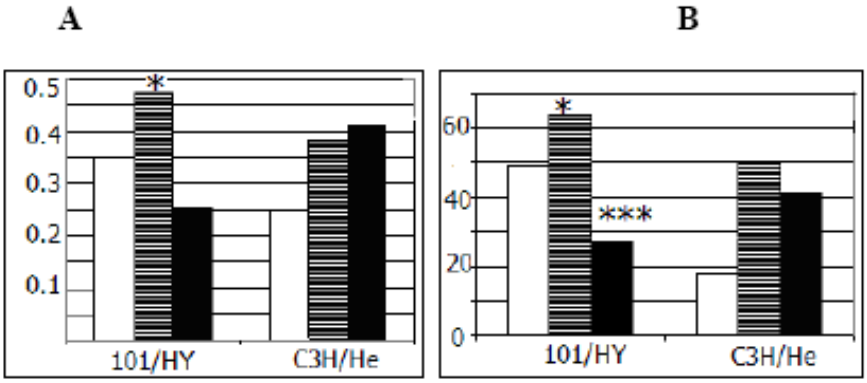


Figure 11. Elevated plus maze test (EPM), mouse experiments. A - number of open EPM entries (ordinate), B – the mean time spent (sec, ordinate) in open EPM arms. Bars - as in Figure 4. *, *** - significantly ($p<0.05$, $p<0.001$) different from intact group scores.

## Extrapolation Task Solution

As the extrapolation test is able to reveal an animal's capacity to solve an elementary logic task in the absence of a similar previous experience, the task solution's scores after its first presentation are a very informative index of this capacity.

Figure 12 A demonstrates the extrapolation task's successful solutions for the first task presentation - the intact animals of both strains correctly solved this task in proportions which were not significantly different from the 50% chance level. In contrast, the respective proportions of correct solutions for mice after neonatal treatments demonstrated much higher levels of correct choices.

As the experimental groups of animals used in this experiment were not numerous, the increased levels of the task's successful solutions were significant for neonatal L-NAME and fluoxetine groups in C3H/He mice and for the fluoxetine group in 101/HY strain only, although the tendency of this variable to be higher in other treatment groups was also present. Nevertheless, the interstrain differences of these scores in the effects of neonatal treatments in both strains were revealed; the remote effects of neonatal treatments being more clear cut in the C3H/He strain in comparison to the 101/HY strain.

The remote effects of neonatal treatments were not so evident in the scores of multiple extrapolation task presentations, indicated by the summarized data for 6 trials.

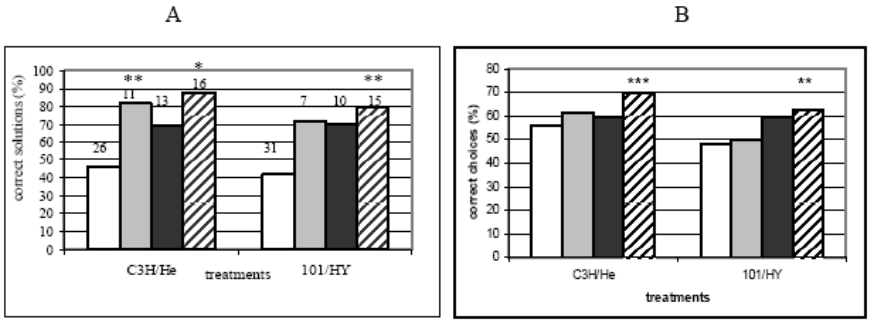


Figure 12. Extrapolation task solutions at first task presentation in 101/HY and C3H/HE mice. Bars as in Figure 4, oblique hatching – neonatal fluoxetine injections (see text). A- first task presentation, the digits above the bars –number of animals in the group. *, **, *** - significantly different from the 50% chance level at $p<0.05$, 0.01 and 0.001, respectively (φ –Fisher method).

Nonetheless, the Nonetheless, the neonatal fluoxetine group demonstrated higher extrapolation scores than did the intact mice, the difference from the 50% chance level being significant for this group (Figure 12, B).

Thus, parallel with the changes in cell proliferation in the SGZ in the rat and mouse dentate area, behavioral changes were found in seizure proneness, anxiety, exploration and in the capacity to solve a cognitive task. Genotype differences in these effects were also present.

# Discussion

The experimental data presented in this chapter evidenced that both neonatal treatments (semax and L-NAME) induce changes in the number of newborn cells in the SGZ and are accompanied by behavioral changes later in life (age 1-2 months). Such changes were noted for audiogenic seizure proneness, open field locomotion, anxiety and exploration indices as well as for the successfulness of a cognitive task solution.

By summarizing the data on cell proliferation in the SGZ of the hippocampal dentate area after neonatal semax and L-NAME treatments in rats and mice, the following conclusion could be drawn: these neonatal treatments lead to an intensification of cell division processes. It is plausible that this increase in cell proliferation could be one of the factors determining the remote behavioral effects of treatments used when they were found during adolescent and adult ages. In this chapter, the data on neonatal semax effects on brain cell proliferation was presented for the first time, whereas the stimulatory effects of neonatal L-NAME had been shown earlier (Peunova, Enikolopov, 1995; Virgili et al., 1999; Matarredona et al., 2003) and confirmed herein.

The increase in new cell numbers in the subgranular area of the hippocampal fascia dentata could be discovered 7-14 days after the injections ceased. Both cell number scores - the total cells as well as the cell'sneuronalcell's neuronal precursors - were increased. Data from the literature claims that a large proportion of newly born cells could be included into neuronal circuits (Kempermann et al., 2003; Nacher et al., 2001). The NOS blockade (by L-NAME) was shown to increase the cell proliferation in both cell cultures and *in vivo* (Packer et al., 2003). As of yet there is no direct evidence concerning neurogenesis changes as the

result of semax injections in adult mice or rats, and our data suggests that such an effect probably exists.

The physiological and molecular mechanisms which determine an increase of brain cell proliferation as the NO levels decrease are to be investigated. At the same time, definite evidence exists which indicates a change in the production of growth factors during these processes and as the result of their modulation (Estrada et al., 1997; Matarredona et al., 2004, 2005).

Changes in audiogenic fit intensity, as the result of neonatal treatments, occurred in mice and rats. The changes in audiogenic seizure proneness, as well as changes in the pattern of seizure fits, found as the remote effects of both neonatal treatments in rats, could have physiological explanations. Changes in seizure patterns could be logically explained by changes in the neuronal excitability of the inferior (as well as superior) colliculi neuronal circuit, crucial for the development of an audiogenic seizure fit. The involvement of the corpora quadrigemina in the development of sound-induced epilepsy was documented earlier elsehwereelsewhere (Faingold et al., 1993, 1994). Keeping in mind that the stimulation of neurogenesis is accompanied by an increase in neurotrophic factor expressions (which induces shifts in the AMP, e.g., element response binding protein phosphorylation) (Xiong et al., 1999; Zhu et al., 2006), it could provoke the changes in audiogenic epilepsy indices. The related events were reported by using another seizure model; NOS inhibition was capable to exert both proconvulsant and anticonvulsant effects (Kirkby, Carroll, 1996).

In spite of the fact that our data demonstrated no interstrain differences in overall cell proliferation, the prevalence of neuroblast formations as a response to a semax neonatal treatment in Wistar, in comparison to KM rats, could be taken into account in explaining the audiogenic fit changes in our experiments. One may suggest that altered cell proliferation and probably the appearance of excessive cell numbers (in comparison to the norm, both for glial and neuronal phenotypes) could determine changes in the functioning of motor centers which participate in the development of audiogenic seizure fits (Faingold et al., 1993, 1994). Audiogenic seizure fit development involves exciting several brain motor centers (Faingold et al., 1993, 1994). Numerous evidence exists that in both SVZ and SGZ cell proliferation was affected by neonatal treatments. One may suggest that altered proliferation and probably the excessive new cells, which were born due to the stimulatory effects of the injected drugs, could cause changes in the functioning of several brain structures as well. Since both unusual remote effects in the audiogenic seizure pattern - the quick cataleptic state development during audiogenic seizure fits and the specific pattern of motor seizures (seizure on the back)- were characteristic for both experimental groups (neonatal semax and L-NAME), it is possible to suggest that not only the brainstem regions participated in audiogenic fit modulation, but basal ganglia structures did as well, particularly the striatum. It was demonstrated (Del Bel, Guimarães, 2000; Del Bel et al., 2005) that “haloperidol” catalepsy, determined to large extent by the dysfunction of the dopaminergic striatal system (blockade of D1-D2 receptors), was enhanced after L-NAME administration to adult animals. This could mean that, in our experiments, temporal NOS inhibition (after neonatal L-NAME) affected the development of neural substrates of the motor seizure and post-ictal catalepsy in rats. Experimental data proves that striatal neurogenesis in the adult brain could be discovered (usually after a brain injury of different origin) (Yang et al., 2008; Zhu et al., 2011; Sun et al., 2012). The SVZ is considered to be a source of cells migrating to the striatuim (Kernie, Parent, 2010), and the respective molecular mechanisms have started to be analyzed (Urbán et

al., 2010). It was shown that clozapine, similar to antidepressants in its action, could increase SGZ neurogenesis (Maeda et al., 2007), whereas haloperidole and a typical antipsychotic could not (Halim, 2004). The functional integration of newly generated neurons into a post-ischemic striatum was described (Hou et al., 2008). These facts are in accordance with our experimental results, namely the changes in audiogenic seizure fit patterns (abnormal posture and catalepsy-like states) as results of the neonatal treatments used. The interstrain differences in changes of AS fits in rats and mice of different genotypes are presently difficult to attribute to definite molecular or biochemical changes, although the differential audiogenic epilepsy proneness in rats and mice of different strains could also be partially responsible for this phenomenon. Unfortunately the neurogenesis modulation by means of semax or by L-NAME early administration was not studied in DBA/2J mice, but the decreased number of animals which did not survive the sound exposure in the group of DBA/2J animals after neonatal semax attracts attention. It could be that the neuroprotective effects of semax, which were already demonstrated in other experiments (Levitskaya et al., 2004; Bashkatova et al., 2001; Umriuchin et al., 2001), were also realized in this case. The audiogenic epilepsy in DBA/2J mice is determined by three chromosomal loci (Newmannm, Collins, 1991), and the changes in AS, which were identified after early semax treatment in this study, could be further analyzed, having in mind this interstrain comparative data.

As already mentioned, an increase in newly born cell numbers as a response to neonatal injections of semax and L-NAME had at least one common link in the chain of processes involved, namely an increase of BDNF (brain derived growth factor) (and probably other neurotrophins) production (Wagner et al., 1999; Dolotov et al., 2006, a, b). The production of NO and the increase of BDNF expressions were shown to correlate with one another, although this data was mostly obtained using cell and tissue cultures (Shadrina et al., 2001; Dolotov et al., 2003; Moreno-Lopez et al., 2004; Agapova et al., 2007; Agasse et al., 2006; Henry et al., 2007). This effect was also shown *in vivo*: semax administration to adult ratsrats' increased BDNF production in the forebrain structures (Dolotov et al., 2003, 2006 a, b). Semax injections after an experimental brain ischemia prevented a two-fold increase in the brain NO levels and the development of ischemic neurological symptoms (Bashkatova et al., 2001; Fadiukova et al., 2001). It is rather difficult to follow the relationship of the two processes (neurogenesis and BDNF production), as data is sometimes contradictory (Xiong et al., 1999; Rossi et al., 2006). The inhibiting action of BDNF on the sodium channel Kir3 (Rogalski et al., 2000) could be one of the mechanisms by which this neurotrophic factor influences postnatal neurogenesis. There were numerous indications that antidepressants intensified cell proliferation in the brain (e.g. Malberg et al., 2000; Kempermann, 2002; Hashimoto et al., 2004; Encinas et al., 2006; Thakker-Varia et al., 2007), and the peripheral BDNF produced the antidepressant effects (Shmidt et al, 2010). Neonatal fluoxetine, which changed the behavior of adult mice in the extrapolation test, also was shown to induce changes in postnatal neurogenesis (Encinas et al., 2006, Hodes et al., 2010). Thus, the whole complicated pattern of NO production, brain growth factor expressions and the pattern of the main neurotransmitter systems' functioning in different brain areas are far from being fully understood (Cameron et al., 1995; Bagni et al., 2002; Dawirs et al., 1998; Banasr et al., 2001; Rattiner et al., 2004; Chen, Russo-Neustadt, 2007; et al.). This regulatory system is very complicated and external factors, such as environmental enrichment, neurotrophin production and stress-related physiological changes, are interconnected affecting brain development (e.g. Adlard, Cotman, 2004; Conti et al., 2002). It was also claimed that nitric oxide acts in a

positive feedback loop with the BDNF, which provides a regulation of the brain cell proliferation process (Chen et al., 2003). The data started to accumulate concerning the possible ways of how genetic differences in neurotrophine effects are realized (Chen et al., 2006). As an example, the genetic differences in adult mice's behavior were demonstrated after an EGF neonatal administration (Tohmi et al., 2005).

The acute and remote effects of semax and NO on behavior have been described earlier. When newborn rats were made to inhale NO over 5 hours, it was further accompanied by delays in the senso-motor development of the pups (Koeter and Rodier, 1986). The increasing or decreasing NO levels and their associated effects on the behavior of laboratory rats were described in an extensive review (Kamensky and Savelieva, 2002). The increase of NO levels in adult brain tissues increased the anxiety of the rats (Roohbakhsh et al., 2007) and mice (Volke et al., 1997; Li et al., 2003, et al.). L-NAME injections increased anxiety in adult mice, judged by the decrease of exploration scores in EPM open arms. However, if the mice's anxiety had decreased after stress-inducing treatments, such an effect was not found (Czech et al., 2003; Pokk and Väli, 2002). The involvement of neurotrophins (and hence neurogenesis) was hypothesized as an important part of the stress model (Duman and Monteggia, 2006).

The differential effects of semax injections were noted in rats with different predispositions to emotional stress (Umriukhin et al., 2001); the exploration activity of rats also changed in response to chronic semax administration (Vilensky et al., 2007). The remote effects of neonatal semax in randomly bred albino rats were described, and the decrease in exploration and increase in learning success were noted (Sebentsova et al., 2005 a, b). The noothropic action of neonatal semax, L-NAME and fluoxetine was demonstrated in our experiments in the elementary reasoning task test. The adult animals which were exposed to these drugs during the first week of life were more successful in solving the extrapolation task when the mouse had to determine the direction in which the food disappeared and how to approach the food's new location. The same (genotype dependent) increase in the extrapolation task's success was shown as the effect of the new nootropic dipeptide drug, Noopept (Bel'nik et al., 2009), shown to induce an increase in the brain's BDNF production (Firstova et al., 2009). Neonatal fluoxetine effects were not analyzed here at the level of cell proliferation, although this drug was shown to increase the number of new cells in mice (Encinas et al., 2006; Hodes et al., 2010).

In our previous experiments, the remote effects of a native ACTH 4-10 fragment and semax neonatal injections in mice of different strains were also demonstrated. Changes in adult animal behaviors were noted which could be put in correspondence with changes in brain monoamine levels and in the number of catecholaminergic neurons, detected earlier in the zona incerta. It should be mentioned that this morphological effect was analyzed in 4.5-month-old animals (Shilova et al., 1996, 2000; Boyarshinova et al., 2004 a). Our experiments demonstrated the remote effects of not only semax, but of neonatal ACTH $_{4\text{-}10,}$ ketamine, pyracetame, caffeine and melatonine (Poletaeva et al., 1996 a; Shilova et al., 2004; Salonin et al., 2004; Markina et al., 2008).

The behavioral indices of the open field test changed in rats and mice as the remote effects of neonatal treatments. These results could be interpreted as the (genotype dependent) change in brain substrates associated with anxiety behavior and/or exploration propensity. The locomotion level in the arena's periphery decreased in Wistar rats over the course of the 3 minute test (habituation) whereas no such habituation was found in intact KM rats. In contrast with the intact KM rats, animals of this strain demonstrated the habituation of the

locomotion scores during the open field test after neonatal treatments, as it was characteristic for all Wistar rat groups and which corresponds to a "normal" habituation process. Thus, the KM rat reaction to novelty, being (abnormally) exaggerated in intact animals, showed habituation in both treatment groups. Neonatal semax elevated, and neonatal L-NAME decreased, the level open-field locomotion in the Wistars. This contrast in both drug treatments' remote effects were not found in KM rats – both had similar remote effects. Thus, these effects could be caused not by the neurogenesis *per se* (the new cells born), but by the strain-specific processes which followed.Numbersfollowed. Numbers of arena squares crossed in the center largely increased in the Wistar neonatal semax group in comparison to the intact group of this genotype and with the KM strain scores. At the same time, the remote effects of neonatal L-NAME were opposite in sign, a significant decrease of squares in the center in both strains (at 2 and 3 minutes of the test) took place. This decline could be explained by the exaggerated habituation of this behavior, or by the specific increase in the level of animal anxiety. Both explanations are preliminary and such phenomena should be analyzed in more detail.

The data on exploration activity (rearing numbers) in Wistar rats were in accordance with the plausible fear-anxiety increase after a neonatal treatment of L-NAME. The number of rearings was high after neonatal semax (as the square numbers in the center were also) and low in the L-NAME treated group. At the same time, the data on KM rats revealed a decrease of exploration (fewer rearings) in both neonatal treatment groups. As in the case of open field periphery locomotion, practically no habituation in the number of rearings occurred in KM rats. Moreover, the low level of locomotion of intact KM rats in the arena's periphery (see Results) may attributed to the high rearing activity of these animals (Figure 8), as less time is left for an animal to move along as "something" drove it to stay on its hind legs. The neonatal semax and L-NAME groups of the KM strain developed less rearings and demonstrated a certain habituation (a decrease of their numbers). The open field exploration (number of rearings) was higher in the KM strain as well as the indices of fear-anxiety behavior. This discrepancy, according to the usual "logic" of laboratory animal behavior interpretation (less anxiety- more rearings), should be taken in mind as it could reflect changes in the brain substrate of these reactions as the result of neonatal treatments.

The EPM test data also demonstrated an increased exploration in KM intact rats, in comparison to Wistars, and an increase of both anxiety indices after neonatal treatments. The time spent in the open arms of EPM, and the number of explorative "hangings over" from open EPM arms are reliable indices of the anxiety level in rodents. Thus, one may suggest that, in general, the neonatal treatments used (semax and L-NAME) induced the increase of animal anxiety. As mentioned above, such analytic considerations create a contradiction: the treatments used in these experiments increased both - the scores of explorative rearings and scores of anxiety markers. As KM rats represent a genetic model for the high predisposition to audiogenic seizures, the excessive "production" of certain fixed action patterns (e.g. rearings), which are normally characteristic for exploration or anxiety, could be ascribed to the abnormal excitability of these animals, although this issue needs special and more profound attention.

The changes in open field and EPM behavior indices in two mouse strains, which were found in groups after neonatal semax, were also obvious from our data. Although in C3H/He mice (neonatal semax), the number of squares crossed in the open field arena reliably increased, and practically no changes in this index occurred in 101/HY mice (Figure 10).

Previous experiments (Shilova et al., 2004) demonstrated that saline injections (neonatal pain stimulation) were found to be a potent treatment which changed the anxiety indices in adult mice of several genotypes, and this was confirmed in experiments presented in this chapter. The neonatal injections of semax "normalized" these changes (which were of opposite directions in the rearing numbers of two strains, see Results). The decrease of open arms entries of the EPM and time spent there was also characteristic for mice groups after neonatal semax.

The behavioral changes in rats and mice as remote effects of neonatal treatments could not be directly explained by changes in neurogenesis intensity. Neither our data nor present information from the literature could indicate which processes are involved in the detail (confirming or denying the causal relationships); extremely complicated processes of brain development are not well understood. The interstrain differences in mouse and rat pain sensitivity, as well as in AS proneness, as remote effects of neonatal semax were already described before (Boyarshinova et al., 2004, 2008; Alexeev et al., 2003; Salonin et al., 2004). The changes in behavior indices as remote effects of neonatal drug injections could be of different signs in comparison to the effects that the administration of thesedrugsthese drugs could have in adult animals (Shilova et al., 2004). It is possible that neonatal treatments could influence the receptor affinity and/or number of different brain neurotransmitter receptors. In turn, these shifts could be the cause of behavioral differences later in life. As these effects could be realized via the multistage neurochemical cascades, genotypic differences could arise. The latter were demonstrated in our earlier studies (Shilova et al., 2004; Boyarshinova et al., 2004 a, b) as well as in the present chapter. As already discussed above, some changes in the behavioral scores of KM and Wistar rats had the opposite sign in two genotypes after neonatal treatments (the significant interaction of factors being revealed by ANOVA, see Results). For example, the locomotion level in the open field arena's center depended on gender, genotype and treatment. The gender differences at the level of adult brain neurogenesis (Zhang et al., 2008) could, at least partly, explain the sexual dimorphism demonstrated.

A significant effect associated with the genotype and treatment factors, which were shown in the data presented in this chapter, reflected the fact that neonatal semax did not have a remote effect in KM rats in several behavior indices, but this treatment significantly increased the scores (e.g., squares crossed in the arena's center) in Wistars. As was mentioned above, both drugs neonatally introduced increased the neurogenesis in two strains of rats and in two strains of mice, and this "discrepancy" could signify that a differential reaction to drugs in developing Wistar and KM brains took place. These differences could arise due to the processes which were not directly connected to the excessive numbers of new cells, but could be determined by other genotype dependent differences, e.g., in neurotrophin production or genetically determined differences in other signaling cascades.

## Conclusion

The neonatal administration of neuropeptide - synthetic ACTH 4-10 fragment analogue - and of the nitrous synthase inhibitor L-NAME was followed by remote effects in two rat and two mouse strains. Changes in audiogenic epilepsy proneness, anxiety and exploration indices

in open-field and elevated plus maze cells were demonstrated. These neonatal treatments, as well as the neonatal administration of the popular SSRI fluoxetine (prozak), had remote effects associated with an increase of successful solutions for elementary logic tasks in mice. The latter effect revealed the nootropic action of these neonatal treatments. As a common feature of all three treatments, one may indicate the increased new cell production at least in the hippocampal subgranular zone (adult neurogenesis). Either more abundant new cells in this brain region appeared, which probably could be incorporated in the functional neuronal circuits, or (which is more probable) the increased production of neurotrophins in the early ontogeny stage of development have the potency to induce changes in the behavior of animals later in life.

The data presented could signify that this approach – the study of remote effects of neonatal treatments, including the analysis of brain morphology, - shows a perspective for plausible treatments of brain anomalies (injury and/or hereditary diseases).

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