# Effect of neonatal dexamethasone treatment on cognitive abilities of adult male mice and gene expression in the hypothalamus

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The early postnatal period is critical for the development of the nervous system. Stress during this period causes negative long-term effects, which are manifested at both behavioral and molecular levels. To simulate the elevated glucocorticoid levels characteristic of early-life stress, in our study we used the administration of dexamethasone, an agonist of glucocorticoid receptors, at decreasing doses at the first three days of life (0.5, 0.3, 0.1 mg/kg, s.c.). In adult male mice with neonatal dexamethasone treatment, an increase in the relative weight of the adrenal glands and a decrease in body weight were observed, while the basal level of corticosterone remained unchanged. Dexamethasone treatment in early life had a negative impact on the learning and spatial memory of adult mice in the Morris water maze. We analyzed the effect of elevated glucocorticoid levels in early life on the expression of the *Crh*, *Avp*, *Gr*, and *Mr* genes involved in the regulation of the HPA axis in the hypothalami of adult mice. The expression level of the mineralocorticoid receptor gene (*Mr*) was significantly downregulated, and the glucocorticoid receptor gene (*Gr*) showed a tendency towards decreased expression ($p = 0.058$) in male mice neonatally treated with dexamethasone, as compared with saline administration. The expression level of the *Crh* gene encoding corticotropin-releasing hormone was unchanged, while the expression of the vasopressin gene (*Avp*) was increased in response to neonatal administration of dexamethasone. The obtained results demonstrate a disruption of negative feedback regulation of the HPA axis, which involves glucocorticoid and mineralocorticoid receptors, at the level of the hypothalamus. Malfunction of the HPA axis as a result of activation of the glucocorticoid system in early life may cause the development of cognitive impairment in the adult mice.
Key words: neonatal dexamethasone treatment; HPA; glucocorticoid receptor; mineralocorticoid receptor; spatial memory; gene expression.

# Влияние неонатального введения дексаметазона на когнитивные способности взрослых самцов мышей и экспрессию генов в гипоталамусе

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Ранний постнатальный период является критическим для развития нервной системы. Стресс в этот период вызывает негативные отдаленные последствия, которые отражаются как на поведенческом, так и на молекулярном уровне. В нашем исследовании для моделирования повышенного уровня глюкокортикоидов, характерного для стрессирующего воздействия в раннем возрасте, мы использовали введение дексаметазона, агониста глюкокортикоидных рецепторов, в понижающихся дозах в первые три дня жизни (0.5, 0.3, 0.1 мг/кг, п/к). У взрослых самцов мышей с неонатальным введением дексаметазона было найдено увеличение относительного веса надпочечников и снижение веса тела, при этом базальный уровень кортикостерона в крови не изменялся. Введение дексаметазона в раннем возрасте оказало негативное воздействие на скорость обучения и формирование пространственной памяти в водном лабиринте Морриса у взрослых животных. Мы проанализировали влияние повышенного уровня глюкокортикоидов в раннем возрасте на экспрессию генов *Crh*, *Avp*, *Gr*, *Mr*, участвующих в регуляции гипоталамо-гипофизарно-надпочечниковой системы (ГГНС), в гипоталамусе взрослых животных. Уровень экспрессии гена минералокортикоидного рецептора (*Mr*) был снижен достоверно, а гена глюкокортикоидного рецептора (*Gr*) – на

Н.П. Бондарь, В.В. Решетников
К.В. Бурдеева, Т.И. Меркулова

Влияние неонатального введения дексаметазона
на взрослых самцов мышей

2019
23•4

уровне тенденции (*p* = 0.058) у самцов мышей с неонатальным введением дексаметазона по сравнению с введением физиологического раствора. Уровень экспрессии гена, кодирующего кортикотропин-рилизинг гормон (*Crh*), не изменялся, тогда как экспрессия гена вазопрессина (*Avp*) повышалась под влиянием неонатального введения дексаметазона. Полученные данные демонстрируют возможное нарушение механизмов негативной регуляции ГГНС на уровне гипоталамуса, в которую вовлечены глюкокортикоидный и минералокортикоидный рецепторы. Нарушение функции ГГНС при активации глюкокортикоидной системы в раннем возрасте может быть причиной развития когнитивных нарушений у взрослых животных.

Ключевые слова: неонатальное введение дексаметазона; ГГНС; глюкокортикоидный рецептор; минералокортикоидный рецептор; пространственная память; экспрессия генов.

## Introduction

As is well known, the early postnatal period is the most important for the development of the central nervous system and of the behavioral phenotype (Teicher et al., 2016). In particular, clinical trials have demonstrated that stressful events in early childhood may have adverse effects on the individual's cognitive and emotional functions at different stages of life, including in adulthood (Pervanidou, Chrousos, 2018; Weems et al., 2018). Similarly, studies in rodents have associated early postnatal stress with some behavioral and cognitive deficits in adult animals (Ladd et al., 2000; Lehmann, Feldon, 2000; Schmidt, 2010). Because the hypothalamic-pituitary-adrenal (HPA) axis plays a key role in stress response, it is surmised that the long-term effects of stress may be associated with persistent changes in the functionality of different molecular constituents of this axis (De Kloet, 2013; Pervanidou, Chrousos, 2018).

According to the classic interpretation, HPA activation in response to stress starts with an increase in the expression levels of the *Crh* and *Avp* genes in the paraventricular nucleus of the hypothalamus (PVN). The products of these genes – corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) – stimulate the expression of the *Pomc* gene and the secretion of adrenocorticotropic hormone (ACTH) by the adenohypophysis (Harno et al., 2018). ACTH, in turn, stimulates the synthesis of glucocorticoid hormones in the adrenals (van Bodegom et al., 2017; Gjerstad et al., 2018). In the brain, the action of these hormones is mediated by glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) (De Kloet, 2013). GRs are expressed in all brain structures, peaking in CRH neurons of the hypothalamic PVN and in adenohypophyseal corticotrophs (Sapolsky et al., 1983; van Eekelen et al., 1991). It is known that, by inhibiting *Crh* and *Pomc* expression (Drouin et al., 1993; Malkoski, Dorin, 1999), activation of GRs in these cells triggers a negative feedback mechanism that reduces HPA activity and terminates the response to the received hormonal signal (McEwen et al., 1992). MR expression is largely confined to the limbic system, peaking in the hippocampus (Sapolsky et al., 1983; van Eekelen et al., 1991). It is surmised that MRs like GRs participate in the control of HPA activity, mediating "proactive" feedback involved in the maintenance of its basal level and in the control of the inhibitory hippocampal effect on HPA function (Berardelli et al., 2013). Additionally, it is well known that GRs and MRs are involved in the formation of cognitive functions, emotional reactions and behavioral reactions (De Kloet, 2013; Paul et al., 2015).

Glucocorticoid levels can be changed by exposure to stressful factors at early ages or to drugs with effects on HPA ac-

tivity. One of the most popular models of early-life stress is prolonged separation of pups from their mothers (maternal separation for 3 h once a day) within the first two weeks of life. As was established in rodent experiments, this kind of stress leads to substantial behavioral and cognitive impairment in adults animals (Pryce, Feldon, 2003; Aisa et al., 2007; Suri et al., 2013; Bondar et al., 2018) and has delayed effects on the expression of stress-response genes (Reshetnikov et al., 2018b). Studies exploring the effects of this kind of stress on HPA gene expression revealed that adult animals have reduced *Gr* expression in the hippocampus (Ladd et al., 2004; Aisa et al., 2007), frontal cortex (Navailles et al., 2010) and striatum (Wong et al., 2015) and enhanced *Avp* expression in the PVN of the hypothalamus (Sanchez et al., 2001; Ladd et al., 2004; Murgatroyd et al., 2009). Our previous work showed enhanced *Avp* expression in the hypothalamuses of adult mice who had experienced maternal separation during the early postnatal period. Additionally, these animals were found to have reduced *Crhr1* mRNA levels in the hippocampus and an increased Mr/Gr mRNA ratio in the hippocampus and hypothalamus (Reshetnikov et al., 2018b).

Administration of the synthetic glucocorticoid hormone dexamethasone during the first postnatal days is another tool used for modeling early postnatal stress in rodents (Wong et al., 2015; Yates et al., 2016). Behavioral deficits in animals like these have been well studied by rat experiments. Those animals were shown to have changes in anxious and depression-like behaviour, learning and memory deficits (Kamphuis et al., 2004; Neal et al., 2004; Claessens et al., 2012; Vazquez et al., 2012; Ko et al., 2014). A few works on mice that use the dexamethasone model have revealed changes in anxious behavior and impairment in novel object recognition (Li et al., 2014a). Although there is a large number of works that explore the effects of neonatal administration of glucocorticoids, delayed effects of these hormones on stress-response genes are not sufficiently well studied – and to a still lesser extent so in mice. Some works demonstrated delayed effects of this "pharmacological" stress on separate HPA genes: a reduction in amount of striatal GR in mice (Wong et al., 2015) and reduced hippocampal *Gr* expression in rats (Vazquez et al., 2012). However, the hypothalamus, this brain's region with importance for HPA function, remains to be poorly studied in the context of the delayed effects of the neonatal rise in glucocorticoids. In this work, the delayed effects of HPA activation during animals' early life were explored using a pharmacological approach, which included the direct activation of the glucocorticoid system by administration of the synthetic glucocorticoid dexamethasone.

The aim of this work was to assess the impact of neonatal dexamethasone administration on cognitive function in adult mice and to trace the delayed effects of this drug on the expression of key genes regulating HPA function at the hypothalamic level. A comparison of original data with experimental data on delayed effects of early-life stress (maternal separation) (Reshetnikov et al., 2018b) will allow the similarity and differences between these two types of exposure to be assessed.

## Materials and methods

**Animals.** The experiment animals were C57Bl/6 mice. The animals were kept under standard conditions in the conventional animal facility of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences (RFMEFI62117X0015) (Novosibirsk, Russia) with a 12-hour light and 12-hour dark cycle. Food and water were available *ad libitum*. All animal procedures were performed in compliance with the EU Council Directive 86/609-EEC and were approved by the Ethics Committee at the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences (protocol No. 39 of September 27, 2017).

**Experimental Design.** Animals started to receive dexamethasone (KRKA, Slovenia) on postnatal day 1 (PND1), the day of birth being designated as PND0. Dexamethasone injections were administered according to the following scheme: 0.5 μg/g body weight on PND1, 0.3 μg/g body weight on PND2, and 0.1 μg/g body weight on PND3. The control group received injections of saline solution (10 μl per animal). All injections were administered subcutaneously once a day between 9 am and 10 am. The administration scheme and doses were chosen according to previous research (Kamphuis et al., 2003; Ko et al., 2014; Li et al., 2014a, b).

On PND30, pups were weaned and grouped by sex and litter before testing. A further experiment involved only males. All experimental procedures were run on adult mice (~PND90). Two cohorts of animals were used for measuring gene expression and assessing cognitive function. Animals in the first cohort (six neonatally injected with saline and six neonatally injected with dexamethasone) were sacrificed by rapid decapitation, blood was collected, the adrenals were removed and weighed, the hypothalamuses were removed and frozen at –70 °C. To obtain serum, blood was kept at room temperature for 1 h and then centrifuged for 10 min at 3000 g; serum was collected and stored at –70 °C until used. The relative adrenal weight was calculated as the ratio of the total weight of two adrenals (in milligrams) to the body weight (in grams). Animals in the second cohort (17 neonatally injected with saline and seven neonatally injected with dexamethasone) were tested in the Morris water maze.

**The Morris water maze test.** This test assesses animals' ability to develop and maintain spatial memory (Morris, 1984; Vorhees, Williams, 2006). A Morris water maze is a pool 100 cm in diameter, with four equally spaced visual cues on the pool wall to mark four quadrants with different starting locations (Target, Opposite, Sector 1 and Sector 2). A platform 10 cm in diameter was set in Target sector, slightly beneath the water. Water in the pool was made opaque with the addition of powdered milk, to keep the platform unseen. The water temperature was 24 ± 1 °C.

Testing lasted five days. First, mice were trained for four consecutive days and received four training trials per day, a total of 16 training trials, each starting at the same time of day. In each training trial, a mouse was placed in one of the sectors and allowed to search for the hidden platform for 1 min. If the mouse did not find the platform within 1 min, the experimenter led the animal to it. After the platform was located, the mouse was left on it for 15 sec to memorize the spatial cues. After that the mouse was taken out and placed in a cage for 15 sec for resting before the next trial. Throughout the experiment, the platform remained at its original position. Finally, on day 5, a probe trial was given: the platform was removed, the mouse was placed in the Opposite sector and the time spent in each sector within 1 min was measured. The test was recorded and processed using EthoStudio software (Kulikov et al., 2005).

**RNA extraction and real-time PCR.** RNA was extracted from frozen tissue specimens using TRIzol Reagent (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. RNA was purified using paramagnetic RNAClean XP beads (Beckman Coulter, Germany) and dissolved in double-distilled water. RNA quality and quantity were evaluated using a NanoDrop 2000 spectrophotometer. Complementary DNA (cDNA) was synthesized using kits produced by Syntol (Russia). The reaction included 1 μg of RNA, all procedures were carried out according to the manufacturer's protocols.

Gene expression was assessed by real-time PCR using the CFX96 Real-Time PCR Detection System (Bio-Rad, USA). We assessed the expression of the glucocorticoid receptor genes *Gr* and *Nr3c1*, the mineralocorticoid receptor genes *Mr* and *Nr3c2*, the corticotropin-releasing factor gene *Crh*, and the arginine vasopressin gene *Avp*. PCR outputs were analyzed using the ΔΔCt method and normalized to the expression of the β-actin gene (*Actb*) as a reference gene. The *Avp*, *Gr* and *Mr* amplification products were detected using EVAGreen; the *Crh* amplification products, using TaqMan probes. Primers and probes (Table 1) were designed using Primer BLAST (NCBI) and the PrimerQuest design tool (IDT Technology). The reaction parameters were as follows: 95 °C for 5 min followed by 38 cycles at 95 °C for 10 sec and at 60 °C for 20 sec. After the completion of the PCR reaction for the systems with the intercalating dye EVAGreen, product specificity was assessed by analysis of melting curves. Each reaction was performed in triplicate. The amplification efficiency was from 90 to 110 % for each primer pair.

**Measuring corticosterone concentrations.** Serum corticosterone concentrations were measured by ELISA using a Corticosterone ELISA Kit (Enzo, New York, NY, USA) according to the manufacturer's protocols. Each reaction was performed in duplicate.

**Statistical data processing.** Statistical processing of the Morris water maze test data was performed by ANOVA and Fisher's LSD as a post hoc test. A comparative analysis of the body weight, relative adrenal weight, corticosterone concentrations and gene expression levels was performed using Student's *t*-test. Differences between the groups were considered statistically significant at $p < 0.05$ and showing a tendency to significance at $p < 0.1$. Statistical analyses were performed using Statistica 6.0.

Н.П. Бондарь, В.В. Решетников
К.В. Бурдеева, Т.И. Меркулова

Влияние неонатального введения дексаметазона
на взрослых самцов мышей

2019
23•4

**Table 1.** Forward and reverse primer sequences

| Gene name | | Sequence (5'→3') | | | Product size, bp |
|---|---|---|---|---|---|
| Gr (Nr3c1) | Nuclear receptor subfamily 3, group C, member 1 | For | ATGTATGACCAATGTAAACACA | | 132 |
| | | Rev | GCTCTTCAGACCTTCCTTAG | | |
| Mr (Nr3c2) | Nuclear receptor subfamily 3, group C, member 2 | For | GTGTGTGGAGATGAGGC | | 155 |
| | | Rev | GGACAGTTCTTTCTCCGAAT | | |
| Crh | Corticotropin releasing hormone | For | GGAGAAGAGAGCGCCCCTAA | | 152 |
| | | Rev | AAGAAATTCAAGGGCTGCGG | | |
| | | Probe | 5,6-ROX-ATGCTGCTGGTGGCTCTGTCGTCC-3BHQ-2 | | |
| Avp | Arginine vasopressin | For | TCTCCGCTTGTTTCCTGAGC | | 230 |
| | | Rev | GGGCAGGTAGTTCTCCTCCT | | |
| Actb | Beta-actin | For | TATTGGCAACGAGCGGTTCC | | 140 |
| | | Rev | TGGCATAGAGGTCTTTACGG | | |
| | | Probe | 5,6-ROX-CCAGCCTTCCTTCTTGGGTATGGAATCC-3BHQ-2 | | |

## Results

**The body weight, relative adrenal weight and corticosterone concentrations.** In the neonatal dexamethasone-treated group, the body weight was lower [$t$ (1, 35) = 4.54, $p < 0.001$], the relative adrenal weight was higher [$t$ (1, 35) = 2.26, $p = 0.030$], and corticosterone concentrations did not differ [$t$ (1, 10) = 0.84, $p = 0.419$] compared to the respective variables in the saline-treated group (Fig. 1).

**The Morris water maze test.** Our studies in adult animals neonatally injected with dexamethasone and given this test showed that administration of this drug at early ages hindered learning in the training trials and adversely affected visual cue-based spatial orientation performance in the probe trial. The repeated measures ANOVA revealed an effect of the factor 'drug' on latency to find the platform in the Morris water maze test. Mice neonatally injected with dexamethasone showed a longer latency to find the platform compared to the controls: trial 7 [F(1, 24) = 7.65, $p = 0.011$], trial 9 [F(1, 24) = 6.48, $p = 0.018$], trial 10 [F(1, 24) = 10.08, $p = 0.004$], trial 11 [F(1, 24) = 10.18, $p = 0.004$], trial 12 [F(1, 24) = 10.74, $p = 0.003$], trial 13 [F(1, 24) = 5.02, $p = 0.034$], and trial 14 [F(1, 24) = 4.63, $p = 0.042$] (Fig. 2, a).

The probe trial, with the platform removed, was given 24 h after the last training trial. Memory acquisition was considered to be displayed if there was a preference for the Target sector compared to any other sector. ANOVA revealed differences in time spent in different sectors by each mouse in the saline-treated group [F(3, 68) = 5.25, $p = 0.003$] and the lack of differences in preferences for sectors shown by any mouse in the neonatal dexamethasone-treated group [F(3, 28) = 0.71, $p = 0.549$]. Mice in the saline-treated group displayed a preference for the Target sector compared to any other sector (Target sector vs Sector 1, $p = 0.013$; Target sector vs Opposite sector, $p = 0.006$; Target sector vs Sector 2, $p = 0.001$). Mice administered with dexamethasone at early ages displayed no preference for the Target sector and spent equal amounts of time in all water maze sectors, suggesting impaired memory acquisition (Fig. 2, b).

**Changes in the hypothalamic expression of genes involved in the glucocorticoid system.** We assessed the expression of the main genes involved in the glucocorticoid
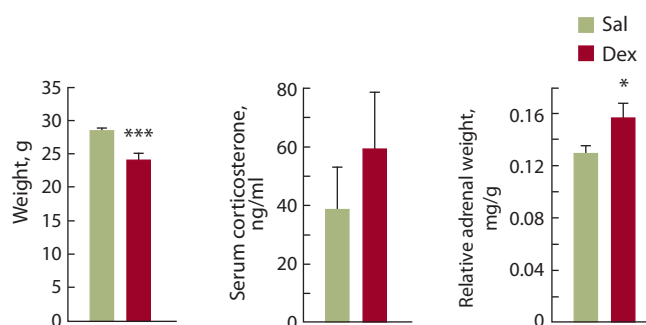


**Fig. 1.** Effects of neonatal dexamethasone treatment on adult mice's body weight, corticosterone concentrations and relative adrenal weight.

Sal, saline-treated group; Dex, dexamethasone-treated group. * $p < 0.05$; *** $p < 0.001$ compared with the Sal group.

system: the glucocorticoid receptor gene, mineralocorticoid receptor gene, corticotropin-releasing factor gene, and arginine vasopressin gene *Avp*. The expression levels of both glucocorticoid (*Gr*) [$t$ (1, 8) = 2.21, $p = 0.058$, a tendency] and mineralocorticoid (*Mr*) [$t$ (1, 8) = 3.28, $p = 0.011$] receptors were lower in dexamethasone-treated mice than in the controls; however, the ratios of the mRNA of these receptors (Mr/Gr) did not differ between the groups. The expression level of *Avp* was higher in dexamethasone-treated mice than in the controls [$t$ (1, 8) = 2.47, $p = 0.039$], while the expression level of *Crh* was unaffected (Fig. 3).

## Discussion

Stress- or glucocorticoid-induced increased HPA activation during early life leads to substantial changes in adult animals' brain structures and behavior. Our study demonstrated that administration of the GR agonist dexamethasone to mice during their first days of life leads to impairment in spatial memory in their adulthood. Along with this impairment, a change in HPA activity was observed in the form of a decrease in the hypothalamic expression of the *Gr* and *Mr* genes in adult animals. GRs and MRs are involved in the regulation
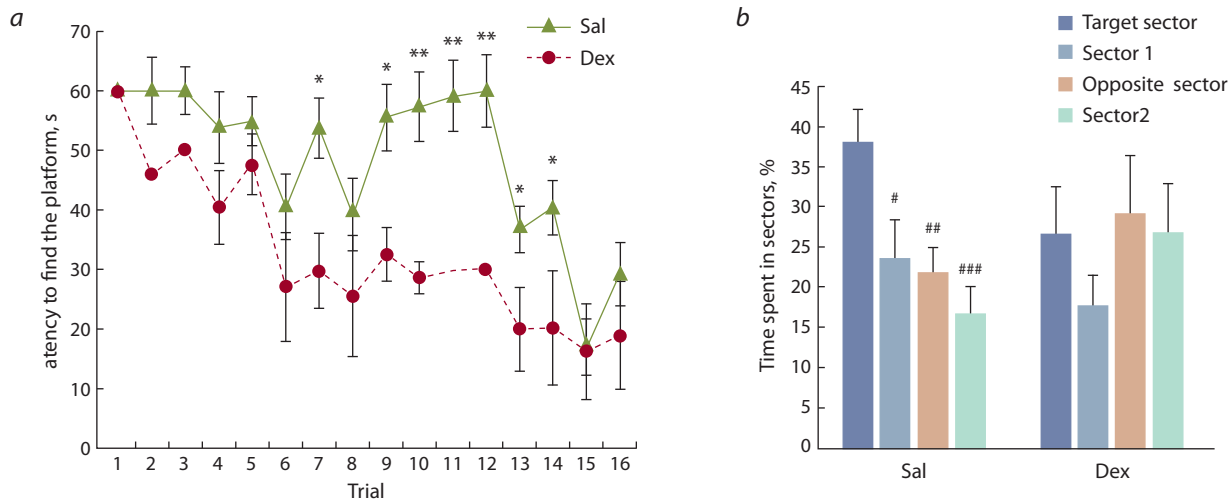
**Fig. 2.** Effects of neonatal dexamethasone treatment on the behavior of adult mice in the Morris water maze. *a*, Latency to find the platform in 16 daily training trials; *b*, Time spent in the each sector in the probe trial (data are presented as the percentage of the probe trial time).

Before the probe trial, the hidden platform was removed, and the mice were placed in the pool for 1 min. Sal, saline-treated group; Dex, dexamethasone-treated group. * $p < 0.05$; ** $p < 0.01$ compared with the Sal group; # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$ compared with the time spent in the Target sector (Fisher's LSD as post hoc).
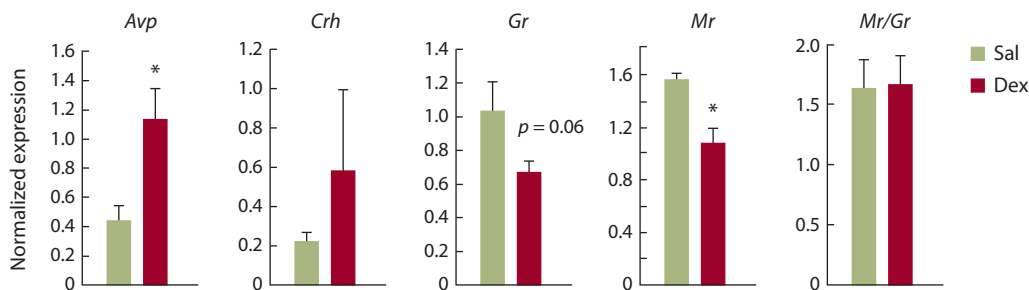


**Fig. 3.** Effects of neonatal dexamethasone treatment on hypothalamic gene expression in adult mice.

Expression levels of *Avp*, *Crh*, *Gr*, and *Mr* mRNA were normalized to *Actb*. Sal, saline-treated group; Dex, dexamethasone-treated group.
* $p < 0.05$ compared with the Sal group.

of stress response and mediate negative feedback during HPA activation, so decreases in their expression suggest a possible disruption in negative regulation of the hormonal response in the hypothalamus.

As is known, the effects of exogenous glucocorticoids strongly depend on the animal species, HPA axis maturity and drug dosage. Adult rats administered with dexamethasone at high doses on postnatal day 5–10 demonstrate reduced neophobia and anxiety in the Light/Dark Box and the Open Field tests, without change in locomotor activity (Yates et al., 2016). At the same time, administration of dexamethasone at low doses to rats during the first three days of their life can cause increases in anxious behavior (Neal et al., 2004; Vazquez et al., 2012) as well as in depressive-like behavior (Ko et al., 2014; Li et al., 2014b). Or, conversely, it may have no effect on the locomotor activity, anxious and social behavior of rats in adulthood (Kamphuis et al., 2004). A few studies involving administration of dexamethasone to mice during the first three days of their life demonstrate that adult ICR strain mice administered with dexamethasone at low doses during the first three days of their life display increased anxiety, while their locomotor and exploratory activities remain unaffected

(Li et al., 2014a). By contrast, neonatal low-dose dexamethasone administration to C57Bl6 strain mice demonstrated a decrease in anxious behavior in adult animals (Batluk et al., 2018), suggesting the strain-specificity of this drug. The most consistently observed and typical effects of both neonatal and prenatal dexamethasone administration that are reported by virtually all researchers working on no matter which animal species are developmental retardation and weight loss (Neal et al., 2004; Lin et al., 2006; Wang et al., 2010; Vazquez et al., 2012; Chiu et al., 2018). We have similar observations: adult mice neonatally treated with dexamethasone had lower body weight compared to the control conspecifics. It is possible that weight loss is associated with a direct effect of dexamethasone on protein catabolism (Weiler et al., 1997; Leret et al., 2004), leading to impairment in skeletal growth (Swolin-Eide et al., 2002).

In this work, we found increases in the relative adrenal weight of adult animals who had been neonatally administered with dexamethasone. Because the corticosterone levels in the adult animals has remained unchanged, the increases in adrenal weight is most likely due to body weight loss, although a possible change in HPA activity induced by

Н.П. Бондарь, В.В. Решетников
К.В. Бурдеева, Т.И. Меркулова

Влияние неонатального введения дексаметазона
на взрослых самцов мышей

2019
23•4

dexamethasone administration during early life may be a factor, too.

In rodent brains, glucocorticoid receptors are mainly located in the hippocampus, especially CA1, and that is why the neurons in this region are most sensitive to glucocorticoids (van Eekelen et al., 1991). As shown on mice and rats, dexamethasone increases caspase-3 activity and apoptosis in the hippocampus and cortex immediately after treatment (Feng et al., 2009; Bhatt et al., 2013; Lanshakov et al., 2016), reduces the number of neurons in the cortex and hippocampus (Kreider et al., 2006; Tijsseling et al., 2013) by PND21, and can also change the hippocampal ratio of the NMDA receptor subunits (Kamphuis et al., 2003), thus changing neuronal plasticity. Neonatal effects of dexamethasone on the hippocampus cause cognitive impairment. Experiments on rats with neonatal dexamethasone administration clearly show a slower learning curve and impaired spatial memory acquisition in the Morris water maze (Ferguson et al., 2001; Kamphuis et al., 2003; Machhor et al., 2004; Qaheri et al., 2013), impaired short-term memory (Claessens et al., 2012), slower learning in passive avoidance tests (Lin et al., 2006; Wang et al., 2010; Chiu et al., 2018) and the poor recognition of familiar and unfamiliar partners (Kamphuis et al., 2004; Wang et al., 2010). As far as mice are concerned, cognitive dysfunctions have been exemplified only with novel object recognition (Li et al., 2014a). Ours is one of the first studies to show impairments inflicted to the learning ability and spatial memory of adult mice by administration of dexamethasone during early life. Mice become slower to learn, as early as on test day 2 they fall behind the control animals in locating the platform. Furthermore, in the probe trial, which was given them 24 h after the last training trial, they did not show preference for the Target sector, where the platform was located, displaying impaired long-term spatial memory.

During the early postnatal period, mice and rats normally have a low stress response (aka hyporesponsive period) because of low blood ACTH and corticosterone concentrations and a reduced number of glucocorticoid receptors in tissues (Levine, 1994). Consequently, most weak stimuli like isolation, a new situation, or saline injection presented during early life did not cause HPA activation. However, powerful stresses like prolonged maternal separation or dexamethasone administration lead to increased HPA activation, which, in turn, causes developmental abnormalities. It is an often occurrence that glucocorticoid administration at early ages does not change basal corticosterone levels in adult animals (Neal et al., 2004; Claessens et al., 2012; Vazquez et al., 2012), which is consistent with our observations, however, what changes is the stress response. Postnatal dexamethasone administration leads to a blunted release of corticosterone in response to a stress being experienced in adulthood (Felszeghy et al., 2000; Flagel et al., 2002; Mesquita et al., 2009; Vazquez et al., 2012), and recovery to basal levels is delayed (Neal et al., 2004).

We assessed the expression of the genes associated with the regulation of HPA activity in the hypothalamus: *Gr*, *Mr*, *Avp* and *Crh*. We demonstrated decreases in the expression levels of the *Gr* and *Mr* genes in mice who had been neonatally administered with dexamethasone. There were no significant differences in the expression levels of the corticotropin-releasing factor gene *Crh* between the groups, however, the expression level of the *Avp* gene, of which the product is involved in the

regulation of ACTH synthesis (Aguilera, Rabadan-Diehl, 2000), was significantly higher in the dexamethasone treatment group. Nevertheless, because of high variances within the groups, there were no differences in basal blood corticosterone levels between the groups, nor do these levels correlate with *Crh* expression levels – although, admittedly, they show a tendency towards an increase. GR levels are directly associated with the dynamics of the hormonal response to stress. As blood corticosterone levels increase, hypothalamic GR activation leads to a reduction in CRH release and, consequently, to a reduction in ACTH release in the hypophysis, normalizing the levels of stress-response hormones (De Kloet et al., 1998). The decrease in hypothalamic *Gr* expression revealed in our work may lead to, among other effects, a decrease in the level of the GR protein itself, which, in its turn, leads to errors in the operation of the mechanisms acting to restore normal levels of hormones after responding to stress. Decreased *Gr* expression levels or decreased amounts of this gene's protein product in the brain's regions are reported in some works on the delayed effects of glucocorticoid administration. Rats neonatally administered with dexamethasone were found to have decreased expression levels of *Gr* in the hippocampus (Vazquez et al., 2012) and a decreased capability of GR to bind the hormone in the hippocampus and hypothalamus (Felszeghy et al., 1996). A reduction in the amount of GR was also found in the mouse striatum after dexamethasone administration during early life (Wong et al., 2015). However, to the best of our knowledge, decreased *Gr* and *Mr* in the hypothalamuses of mice neonatally administered dexamethasone were not shown previously. Therefore, our data add to knowledge about delayed changes in the HPA axis after neonatal dexamethasone administration.

Because administration of dexamethasone during the first days of life can imitate a stressful event experienced at an early age, we compared our new data with those obtained previously on the effects of early-life stress on cognitive functions and hypothalamic gene expression in mice (Table 2) (Reshetnikov et al., 2018b). Unlike neonatal dexamethasone administration, prolonged separation of pups and their mothers during the first weeks of life does not affect the learning curve in the Morris water maze, for the experimental mice learned to locate the hidden platform as rapidly as did their control conspecifics. However, as soon as 24 h later the experimental animals stopped showing preference for the sector where the platform had been set up and then removed, suggesting impaired long-term spatial memory. Thus, more marked cognitive deficits result from neonatal dexamethasone administration than from the stress caused by maternal separation, because the former affects both learning and the reproduction of newly learned information. A comparison of available data on other aspects of cognitive functions shows that impairments in novel object recognition that are due to both early-life stress (Reshetnikov et al., 2018a) and neonatal dexamethasone treatment (Li et al., 2014a) are similar.

Stronger delayed effects of neonatal glucocorticoid administration than of early postnatal stress in the form of maternal separation can be revealed by comparing respective changes in gene expression. Thus, the expression levels of the *Gr* and *Mr* genes were not affected by early-life stress (maternal separation), but were decreased following neonatal dexamethasone administration. Early-life stress led to increased *Mr*/*Gr* ratios

**Table 2.** Comparison of the effects of prolonged separation
of pups from mothers in early life (MS) and the effects
of neonatal treatment with dexamethasone

| Parameters | MS* | Dexamethasone |
|---|---|---|
| Weight | = | Reduced |
| Adrenal glands weight | = | Elevated |
| Level of corticosterone | = | = |
| Level of genes expression in hypothalamus | | |
| Gr | = | Tendency to be reduced |
| Mr | = | Reduced |
| Ratio mRNA Mr/Gr | Elevated | = |
| Avp | Elevated | Elevated |
| Crh | = | = |
| Learning and memory in Morris water maze | | |
| Learning | = | Reduced |
| Long-term memory | Reduced | Reduced |

* Comparison with (Reshetnikov et al., 2018b).

both in the hypothalamus and hippocampus due to a slight
increase in *Mr* expression. This fact explains a weaker effect of
this stress on learning, because the balance of these receptors is
involved in the stress response and the acquisition of long-term
memory (De Kloet, 2013). Neonatal dexamethasone treatment
did not affect the *Mr*/*Gr* ratio: a decrease in the expression
of either type of receptor is accompanied by a decrease in
the expression of the other, without a compensatory change
in their balance that would, if it were present, act to restore
HPA function. The increase in *Avp* expression levels is twice
as high following neonatal dexamethasone administration as
it is following early postnatal stress, while *Crh* expression
remains unchanged, no matter which type of stress is applied.
Thus, neonatal dexamethasone administration leads to stronger
delayed effects on cognitive abilities and hypothalamic gene
expression compared to the stress caused by maternal separa-
tion during the first weeks of life.

## Conclusion
Our results demonstrate that adult mice who have experienced
neonatal dexamethasone administration show a slower learn-
ing curve and impaired spatial memory acquisition in the
Morris water maze. At the same time, adult mice so treated
were found to have decreased expression levels of the *Gr*
and *Mr* genes and increased *Avp* in the hypothalamus, sug-
gesting delayed HPA dysregulation. Early postnatal stress in
the form of maternal separation and postnatal dexamethasone
administration lead to unidirectional changes in the cognitive
abilities and HPA functions of adult animals, however, these
changes are stronger following dexamethasone administra-
tion – probably because these different kinds of treatment
lead to different HPA activation levels.

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