Вавиловский журнал генетики и селекции. 2023;27(6):651-661
DOI 10.18699/VJGB-23-76

Original Russian text https://vavilovj-icg.ru/

The expression profile of genes associated with behavior, stress, and adult neurogenesis along the hippocampal dorsoventral axis in tame and aggressive foxes

Yu.V. Alexandrovich[1], E.V. Antonov[1, 2], S.G. Shikhevich[1], A.V. Kharlamova[1], L.V. Meister[1], Y.V. Makovka[1], D.V. Shepeleva[1], R.G. Gulevich[1] ✉, Yu.E. Herbeck[1, 3]

[1] Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
[2] Sirius University of Science and Technology, Scientific Center for Translational Medicine, Sochi, Russia
[3] Koret School of Veterinary Medicine, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel
✉ gulevich@bionet.nsc.ru

Abstract. The hippocampus plays the key role in stress response regulation, and stress response appears to be weakened in domesticated animals compared to their wild relatives. The hippocampus is functionally heterogeneous along its dorsoventral axis, with its ventral compartment being more closely involved in stress regulation. An earlier series of experiments was conducted with a unique breeding model of animal domestication, the farm silver fox (*Vulpes vulpes*), which included tame, aggressive, and unselected animals. A decrease in many indices of the hypothalamic–pituitary–adrenal activity was observed in tame animals. Also, adult hippocampal neurogenesis was more intense in tame foxes, and this fact may relate to reduced stress levels in this experimental population of foxes. Nevertheless, the molecular mechanisms responsible for the reduced stress response in tame animals remain obscure. In this study, serum cortisol levels and the mRNA levels of 13 genes in the dorsal and ventral hippocampus have been measured and compared in tame, aggressive, and unselected foxes. At the current stage of domestication, stress-induced cortisol levels in tame, aggressive, and unselected animals differ significantly from each other: tame foxes show the lowest levels, and aggressive ones, the highest. Twelve genes tested demonstrate significant gene expression differences between the dorsal and ventral hippocampi. These differences are mainly consistent with those found in rodents and humans. In tame foxes, significantly elevated mRNA levels were recorded for several genes: *CYP26B1* for cytochrome P450 26B1 and *ADRA1A* for α_{1A} adrenergic receptor in the dorsal hippocampus, whereas the level of *NR3C2* mRNA for mineralocorticoid receptor was higher in the ventral. It is presumed that these genes constitute an important part of the mechanism reducing stress induced by contacts with humans and contribute to linking stress regulation with adult neurogenesis in tame foxes and domesticated animals in general.
Key words: tame behavior; aggression; domestication; silver fox; cortisol.

For citation: Alexandrovich Yu.V., Antonov E.V., Shikhevich S.G., Kharlamova A.V., Meister L.V., Makovka Y.V., Shepeleva D.V., Gulevich R.G., Herbeck Yu.E. The expression profile of genes associated with behavior, stress, and adult neurogenesis along the hippocampal dorsoventral axis in tame and aggressive foxes. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding*. 2023;27(6):651-661. DOI 10.18699/VJGB-23-76

Профиль экспрессии генов, связанных с регуляцией стресса, поведения и нейрогенеза, вдоль дорзовентральной оси в гиппокампе у взрослых ручных и агрессивных лисиц

Ю.В. Александрович[1], Е.В. Антонов[1, 2], С.Г. Шихевич[1], А.В. Харламова[1], Л.В. Мейстер[1], Ю.В. Маковка[1], Д.В. Шепелева[1], Р.Г. Гулевич[1] ✉, Ю.Э. Гербек[1, 3]

[1] Федеральный исследовательский центр Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия
[2] Научно-технологический университет «Сириус», Научный центр трансляционной медицины, Сочи, Россия
[3] Школа ветеринарной медицины им. Й. Корета, Факультет сельского хозяйства, продовольственных ресурсов и окружающей среды им. Р.Г. Смита, Еврейский университет в Иерусалиме, Реховот, Израиль
✉ gulevich@bionet.nsc.ru

Аннотация. Гиппокамп является ключевой структурой в регуляции стресс-ответа, который, по-видимому, снижен у домашних животных по сравнению с их дикими сородичами. Известно, что гиппокамп функционально неоднороден вдоль дорзовентральной оси, и в регуляции стресса в большей мере участвует вентральная часть. В серии экспериментов на уникальной селекционной модели одомашнивания животных – серебристо-черной лисице (*Vulpes vulpes*), включающей ручных, агрессивных и неселекционированных животных,

ранее было показано снижение активности гипоталамо-гипофизарно-надпочечниковой системы во многих звеньях. Кроме того, известно, что уровень нейрогенеза в гиппокампе повышен у взрослых ручных лисиц, что может быть взаимосвязано со снижением уровня стресса. Тем не менее молекулярно-генетические механизмы снижения стресс-ответа у доместицированных животных по-прежнему не ясны. В настоящей работе выполнено сравнение мРНК 13 генов в дорзальном и вентральном гиппокампе и проведен анализ кортизола в крови у ручных, агрессивных и неселекционированных лисиц. Установлено, что на данном этапе доместикации стресс-индуцированный уровень кортизола у ручных, агрессивных и неселекционированных животных достоверно отличается друг от друга, причем у ручных животных он самый низкий, а у агрессивных – самый высокий. Выявлены достоверные различия в экспрессии 12 генов между дорзальной и вентральной частями гиппокампа, что в большинстве случаев соответствует аналогичным различиям, найденным у грызунов и человека. У ручных лисиц обнаружен достоверно повышенный уровень в дорзальном гиппокампе мРНК генов цитохрома P450 26B1 (*CYP26B1*) и адренергического рецептора α_{1A} (*ADRA1A*), а в вентральном гиппокампе – мРНК гена минералокортикоидного рецептора (*NR3C2*). Эти гены могут быть важной частью механизма снижения стресса по отношению к человеку и взаимосвязи регуляции стресса и нейрогенеза у взрослых ручных лисиц в частности и доместицированных животных вообще.
Ключевые слова: ручное поведение; агрессия; доместикация; серебристо-черные лисицы; кортизол.

Introduction

The hippocampus is an important brain region involved in the regulation of stress response, learning, spatial memory, social recognition, and memory consolidation. Hippocampus sizes in mammals and birds and adult neurogenesis in mammals are likely to correlate with the capacity for spatial orientation and memory. Also, changes in hippocampus morphology may be associated with adaptive evolution (Jacobs et al., 1990; Jacobs, Spencer, 1994; Rehkämper et al., 2008; Croston et al., 2015; Sonnenberg et al., 2019). However, other scientists presume that neurogenesis rate and hippocampus size, albeit adaptive, are not related to memory or spatial orientation (Lipp, 2017). The 15-fold increase in neurogenesis rate in adult red foxes as compared to dogs (Amrein, Slomianka, 2010) may be related to spatial memorization, typical of foxes hoarding food (Sklepkovych, Montevecchi, 1996). It is known that the CA2 region takes part in social recognition memory (Tzakis, Holahan, 2019). The CA1 and CA3 volumes in primates appear to be related to social and environmental signals, such as group and home range sizes (Todorov et al., 2019).

The hippocampus is among the key elements of the central regulation of the hypothalamic–pituitary–adrenal (HPA) axis. It is known to be functionally and structurally heterogeneous along its dorsoventral axis. It is believed that the regulation of HPA axis action and stress and emotional responses are governed primarily by the ventral hippocampus, and cognitive functions, by the dorsal (O'Leary, Cryan, 2014; Gulyaeva, 2019). This specialization may be due to the locations of these hippocampus compartments, and, correspondingly, the greater number of dorsal hippocampus projections to the crust and of ventral hippocampus projections to regions belonging to the limbic system (O'Leary, Cryan, 2014).

Along with the lateral ventricle subependymal zone, the hippocampus is a region where neurogenesis occurs constantly, even in adulthood (Ming, Song, 2011). The neurogenesis rate is reduced by stress in most cases, and, vice versa, high neurogenesis rate mitigates the effect of stress on the hippocampus (Levone et al., 2015). Thus, stress and the hippocampus functional response exert reciprocal actions. Apparently, the effect of stress on neurogenesis varies along the dorsoventral axis (O'Leary, Cryan, 2014). This inference may explain the fact that the hippocampus neurogenesis rate in tame foxes, whose stress response is much weaker, is higher than in unselected ones, and the most pronounced differences are recorded in the ventral and intermediate compartments of the hippocampi (Huang et al., 2015). Studies on dogs have shown that differences between the dorsal and ventral hippocampi are at the same level despite considerable variations in the overall neurogenesis rate among individuals (Lowe et al., 2015).

Experiments with hippocampus samples from rats, mice, and humans reveal differences in gene expression between the dorsal and ventral hippocampi. They are likely to reflect the functional and structural heterogeneity along the axis (Cembrowski et al., 2016; Lee et al., 2017; Floriou-Servou et al., 2018; Vogel et al., 2020). However, the expression profiles of these genes along the hippocampus dorsoventral axis have not been studied in other taxa, in spite of their functional and structural features.

These data motivated us to investigate gene expression variation along the dorsoventral axis and seek molecular mechanisms linking neurogenesis and stress by quantitation of mRNAs of 13 genes in the dorsal and ventral hippocampi of silver foxes. This species serves as a model of animal domestication. Its "tame" and "aggressive" populations had been raised by long-term selection for friendly or aggressive attitude to humans, respectively, and foxes not subjected to targeted selection for behavior served as control.

These populations differ significantly in many links of their glucocorticoid stress response and HPA axis activity, which seems to be a common feature of domestic animals (Belyaev, 1979; Price, 2000; Trut et al., 2004, 2009), and in adult neurogenesis in the hippocampus (Huang et al., 2015). Genes of the retinoic acid pathway, associated with neurogenesis activity, which are located in regions presumably affected by the selection, have been found in genome-wide

analysis of the tame foxes (Kukekova et al., 2018; Trut et al., 2021). Also, it has been found that the amount of mRNA of one of these genes, *CYP26B1*, in the dorsal hippocampus of tame foxes differs from aggressive ones (unpublished results). This may be one of the mechanisms altering adult neurogenesis in foxes, which can also modulate stress level, learning, memory, and social behavior. For all that, nothing is known about changes in *CYP26B1* mRNA levels in the ventral hippocampi of foxes with contrasting behaviors, although its elevated expression characterizes the dorsal hippocampus in mice, rats, and humans.

We also measured the dorsal and ventral hippocampus levels of mRNAs of other genes related to neurogenesis, stress, or behavior whose expression is known to vary significantly along the dorsoventral axis in mice, rats, and humans (Vogel et al., 2020) (see Table 1). The genes associated with HPA axis regulation include *NR3C1*, *NR3C2*, and *HSD11B1* for glucocorticoid receptors 1 and 2 and hydroxysteroid 11β-dehydrogenase 1, respectively (de Kloet et al., 2016). *NR2F2* is one of the most reliable markers of the position along the dorsoventral hippocampus axis, and it supposedly acts as a mediator of the transcription activity induced by receptors of glucocorticoids and retinoic acid. This action is likely to be associated with relationships between stress and neurogenesis (de Martino et al., 2004; Vogel et al., 2020). The *ADRA1A* gene for the α_{1A} adrenergic receptor is presumed to act in the regulation of behavior and neurogenesis (Doze et al., 2011; Vogel et al., 2020). *KCND2*, *KCND3*, *CADM2*, and *CPNE2* are associated with K^+- and Ca^{2+}-dependent synaptic and glutamatergic transmissions (Corradini et al., 2014; Truvé et al., 2020; Haddjeri-Hopkins et al., 2021; Xiao et al., 2021), which are likely to play the key role in domestication-driven behavior changes (O'Rourke, Boeckx, 2020; Trut et al., 2021). The expression levels of *TRHR*, on the one hand, and *LCT*, *NTS*, on the other hand, have been used as markers of the ventral and dorsal hippocampi, respectively (Cembrowski et al., 2016; Lee et al., 2017).

Earlier data on the glucocorticoid-mediated stress response in foxes demand re-research at the present phase of selection. After over sixty generations of the selection of Norway rats according to approximately the same criteria as foxes as an alternative experimental domestication model, differences between tame and aggressive animals in the glucocorticoid-mediated stress response vanished. This might result from the adaptation of rats selected to aggression toward humans (Prasolova et al., 2014). Besides, previous studies employed nonsocial restriction stress (Trut et al., 2004, 2009). This procedure is less adequate than the use of social stress in studies of animal–animal and animal–human contacts. It has been shown that restriction stress in rats selected for anxiety-like behavior induces a stronger corticosterone-mediated response than in animals selected for low levels of such behavior, whereas social stress in the resident–intruder test shows quite the opposite (Veenema, Neumann, 2007). Here we studied the stress response to a combined treatment: manual fixation of foxes by a social subject, human. Manual fixation for 15 min was a stress factor for all the three fox populations, because foxes had never been picked in arms during selection and they had had an opportunity to avoid close contacts with humans.

Materials and methods

Experimental animals. Experiments were conducted with three experimental populations of silver foxes (*Vulpes vulpes*): tame, aggressive, and unselected. The first two populations had been selected for friendly and aggressive-fearful reactions to humans, respectively, at the Shared Access Center for Gene Pools of Fur and Farm Animals, Institute of Cytology and Genetics, Novosibirsk, for over 60 years (Belyaev, 1979; Trut et al., 2004, 2009).

Blood was sampled from the vena saphena of 6–7-month-old males prior to experimental stress (manual fixation for 15 min) and immediately after it. Naïve (having experienced no experimental stress) 7–8-month old males were euthanized by injections of 5 % sodium thiopental. Fragments of the dorsal and ventral hippocampi were sampled. All samples were stored at –70 °C. Experimental protocols followed the Guidelines for Accommodation and Care of Laboratory Animals, Species-specific Provisions for Laboratory Predatory Mammals, GOST 33217-2014, and Directive 2010/6106/EU for the Protection of Laboratory Animals.

Chromatography. Blood serum cortisol was assayed by high-performance liquid chromatography with an Agilent 1200 Series LC chromatograph equipped with a diode-array detector and a ZORBAX C18 2 × 150 mm × 5 μm column, as in previous studies (Ovchinnikov et al., 2018). Samples were concentrated by liquid extraction with 1,2-dichloroethane. Elution was done with 30 % aqueous acetonitrile at the rate 1 mL/min. Absorption was measured at $\lambda = 246$ nm. Concentrations were calculated against dexamethasone as an internal standard.

Total RNA isolation and real-time PCR. RNA was isolated with TRI Reagent (Molecular Research Center, Inc.) according to manufacturer's recommendations, as in (Ovchinnikov et al., 2018). Absorption values were measured with a NanoPhotometer N50 (Implen, Germany). For RNA purity assessment, A260/A280 and A260/A230 ratios were calculated. Genomic DNA was removed from RNA samples with a DNase I, RNase-free kit (Thermo Fisher Scientific, Lithuania). cDNA was synthesized in a 20 μL volume with 0.2 μg of DNA-free RNA and a Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Fisher Scientific, Lithuania).

Primers for the genes studied were designed using the online web resource Primer-BLAST (Ye et al., 2012). The sequences are shown in Table 1.

Real-time PCR was conducted in a Roche LightCycler 96 Real-Time PCR System (Roche Diagnostics, Switzerland). The reaction volume 20 μL contained 4 μL of twentyfold

Table 1. Primers for real-time PCR

Gene	Primer sequences
NTS	fNTS-F1: 5′-TGGTGTGCATGATACTTCTGG-3′ fNTS-R1: 5′-ACACTTGCTTTGCTGATCTTTG-3′
NR2F2	fNR2F2-F3: 5′-AGCCAAGGAATGTGTCCAAG-3′ fNR2F2-R3: 5′-CAATTCAGGAACTAAGCGGGA-3′
ADRA1A	fADRA1A-F: 5′-CGTGCTCCAGTCAAGAGTTT-3′ fADRA1A-R: 5′-AAGGTATAGCCCAGGGTGTG-3′
CPNE2	fCPNE2-F6: 5′-CCAGGTCATGTGTTACGACT-3′ fCPNE2-R6: 5′-CCTTTGCTTCTTGGGGTTGA-3′
CYP26B1	fCYP26B1-F1: 5′-TTCTTTGGCCTGGACTCGAA-3′ fCYP26B1-R1: 5′-GGCTAGGCGCAGTTAGAC-3′
CADM2	fCADM2-F3: 5′-GTAGCCATAACAACCAGCCC-3′ fCADM2-R3: 5′-AGAACACAGCGTGACAAATACA-3′
KCND2	fKCND2-F1: 5′-GAAAACCTTCCGCATCCCAAA-3′ fKCND2-R1: 5′-ACATTCTTCCATCTTGGCGTT-3′
KCND3	fKCND3-F6: 5′-CAGAGAGCCGATAAACGCAG-3′ fKCND3-R6: 5′-GTGCAGATAGGCATTGGAGC-3′
TRHR	fTRHR-F1: 5′-GACTCAACCCATCAGAACAAGA-3′ fTRHR-R1: 5′-GGGCATCCATAAAAGGGCAA-3
LCT	fLCT-F1: 5′-AAGAACGGCGATTACAACGA-3′ fLCT-R1: 5′-TGCCATTGATCCTCCTCTTCT-3′
HSD11B1	fHSD11B1-F2: 5′-GCAAGGGGATTGGAGAACAG-3′ fHSD11B1-R2: 5′-GGTGCCAGGAATGTAGTGTG-3′
NR3C1	fNR3C1-F6: 5′-CAAGCTGGGATGAACTTGGA-3′ fNR3C1-R6: 5′-AGTTTCTTGTGACGCTCCTG-3′
NR3C2	fNR3C2-F7: 5′-AAAGGCTACCACAGTCTCCC-3′ fNR3C2-R7: 5′-TCATCGGTCCTCTCTGTAGG-3′
CANX	CANX-F1: 5′-GATGCCCCTGCTAAGATTCC-3′ CANX-R1: 5′-CTTCATCCCAATCCTCTGGC-3′

diluted cDNA, 0.3 μL of primers (10 pmol/μL), 7.4 μL of Milli-Q H_2O, 8 μL of 2.5× reaction mix for Real-Time PCR, and SYBR Green I dye (Mfr. Part No. M-427, Syntol, Russia). Each reaction was performed in two technical replications.

The results were processed by the modified $\Delta\Delta C_t$ method (Livak, Schmittgen, 2001) implemented in GenEx ver.6 software (Multi-D, Sweden). This method allows the reaction efficacy to be estimated. The *CANX* gene for calnexin was used as reference, because its expression is high, little variable among individual foxes, and uniform in the dorsal and ventral hippocampi, as confirmed by analysis with NormFinder software (Andersen et al., 2004). The mean expression of each gene was taken to be an arbitrary unit for the evaluation of relative expression. An additional external reference sample was present in all plates for proper comparison of the results obtained in different plates.

Statistical evaluation. The statistical significance of hormone assays in the experimental groups was assessed by repeated measures factor analysis followed by *post hoc* Fisher's LSD test. The increase in cortisol level was assessed by Student's *t* test.

Real-time PCR results were compared by the Kruskal–Wallis test. Pairwise comparisons were done by the Mann–Whitney test. We applied nonparametric criteria, because the samples did not conform to the Gaussian distribution according to the Kolmogorov–Smirnov test.

Use was made of software packages Statistics 10 (StatSoft, United States) and GenEx ver.6 (Multi-D, Sweden). All differences were considered significant at $p < 0.05$. The results are shown in figures as mean ± SEM.

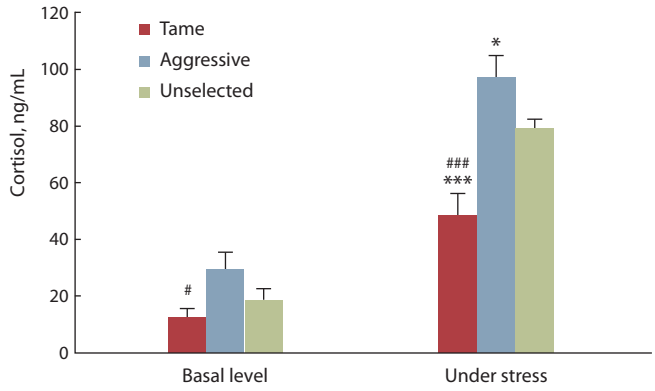

Fig. 1. The basal and stress-induced blood serum cortisol levels in tame, aggressive, and unselected foxes ($n = 11$ in each group).

* $p < 0.05$, *** $p < 0.001$ in comparison with unselected foxes.
\# $p < 0.05$, ### $p < 0.001$ in comparison with aggressive foxes.

Results

Blood serum cortisol in response to stress

Repeated measures factor analysis reveals the effects of genotype ($F_{2.28} = 9.62$, $p < 0.001$) and stress ($F_{2.28} = 179.72$, $p < 0.001$) on blood serum cortisol. The interaction of the *genotype* and *stress* factors was also significant ($F_{2.28} = 9.36$, $p < 0.01$). The basal serum cortisol level in tame foxes was lower than in aggressive (Fig. 1, $p < 0.05$) but did not differ significantly from unselected ones. The increase in serum cortisol level was statistically significant in all genotypes, but it was less pronounced in tame foxes than in aggressive or unselected. Thus, the cortisol level under stress in aggressive and unselected foxes exceeded the value in tame ones ($p < 0.001$, see Fig. 1) and was higher in aggressive foxes than in unselected ($p < 0.05$, see Fig. 1).

Levels of mRNAs in the dorsal and ventral hippocampi of foxes

Quantitative real-time PCR revealed in the dorsal hippocampus significantly higher levels of the genes *HSD11B1*, *CYP26B1*, *CADM2*, *KCND2*, *NR3C1*, *LCT*, and *NR3C2* and in the ventral, *TRHR*, *CPNE2*, *ADRA1A*, and *NR2F2*, whereas *KCND3* showed no difference (Fig. 2, Table 2).

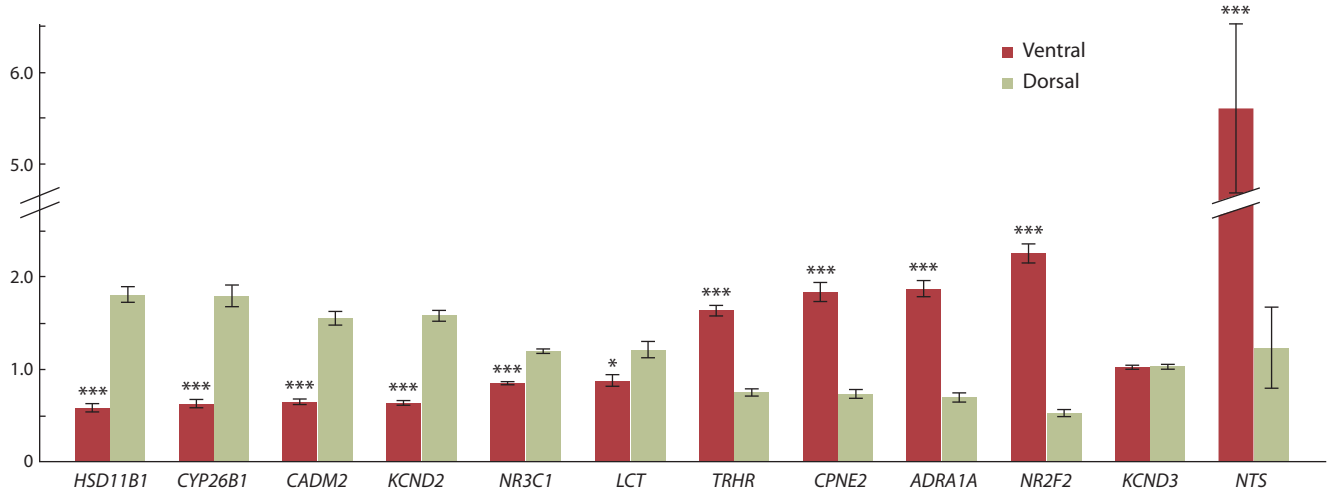

Fig. 2. Differences between the dorsal and ventral hippocampi in relative mRNA levels (n = 18 in each group).

* $p < 0.05$, *** $p < 0.001$ as compared to the dorsal hippocampus.

Table 2. Differences between the dorsal and ventral hippocampi in gene expression in mammals

Species	*HSD11B1*	*CYP26B1*	*CADM2*	*KCND2*	*NR3C1*	*LCT*	*NR3C2*	*TRHR*	*CPNE2*	*ADRA1A*	*NR2F2*	*KCND3*	*NTS*	Reference
Mouse	v (DG) d (CA1)	d (CA3)	d (DG, CA3, CA1)	d (DG, CA3, CA1)	–	d (DG, CA3)	d (DG, CA1)	–	v (CA3, CA1)	v (CA1)	v (CA3, CA1)	v (CA3, CA1)	–	Cembrowski et al., 2016
	d	d	d	d	–	d	d	v	v	v	v	v	v	Floriou-Servou et al., 2018
Rat	–	d	d	d[a]	d[c]	–	d[b]	v	v	–	v	–	d	Lee et al., 2017
Human	v	d	d	–	–	v	–	v	v	v	v	–	d	Vogel et al., 2020
Fox	d	d	d	d	d	d	d	v	v	v	v	–	v	Our data
p	<0.001	<0.001	<0.001	<0.001	<0.001	0.018	0.001	<0.001	<0.001	<0.001	<0.001	>0.05	<0.001	

Note. CA1, CA3, and DG (dentate gyrus) are hippocampus regions; d, mRNA level is higher in the dorsal hippocampus; v, mRNA level is higher in the ventral hippocampus; –, no difference or not known. *p* values are indicated according to the Mann–Whitney test.
[a] 28 and 45 days (growing animals); [b] 28 days; [c] rat *Nr3c1* mRNA according to Kvichansky et al. (2017).

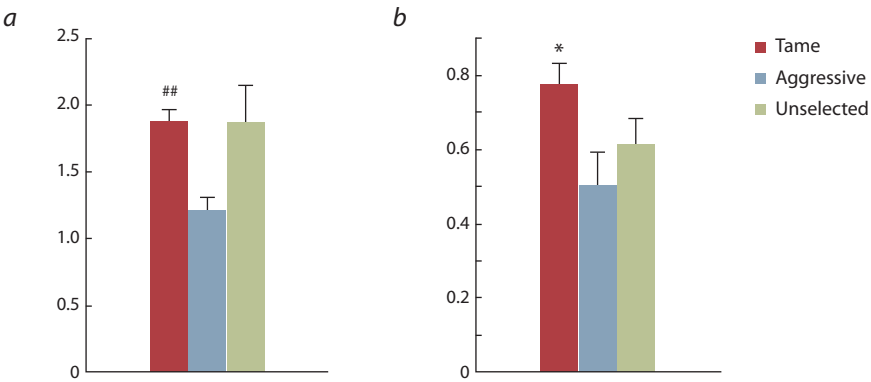

Fig. 3. Relative amounts of *CYP26B1* (*a*) and *ADRA1A* (*b*) mRNA in the dorsal hippocampi of tame (n = 7), aggressive (n = 6), and unselected (n = 6) foxes.

$p < 0.01$ as compared to aggressive foxes; * $p < 0.05$ as compared to aggressive and unselected foxes.

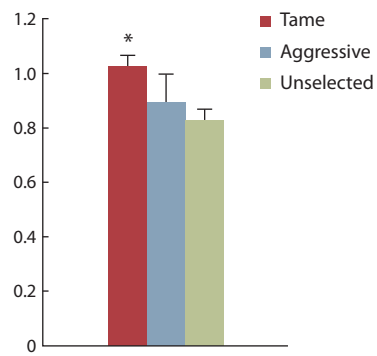

Fig. 4. Relative amounts of *NR3C2* mRNA in the ventral hippocampi of tame (n = 7), aggressive (n = 6), and unselected (n = 6) foxes.

* $p < 0.05$ as compared to unselected foxes.

NTS mRNA levels showed a broad variation among hippocampi of individuals, but the expression in the ventral compartment was always higher (see Fig. 2, Table 2), and so was the neurogenesis rate in a study on dogs reported by Lowe et al. (2015).

Levels of mRNAs in the hippocampi of tame, aggressive, and unselected foxes

We compared the levels of mRNAs indicated in Table 2 in tame, aggressive, and unselected fox groups by the Kruskal–Wallis test and revealed effects of genotype on mRNAs of the genes (1) *CYP26B1* ($H(2, n = 19) = 8.89$; $p = 0.02$) and *ADRA1A* ($H(2, n = 19) = 7.81$; $p = 0.02$) in the dorsal compartment and (2) *NR3C2* for a mineralocorticoid receptor ($H(2, n = 19) = 7.07$; $p = 0.03$) in the ventral compartment. Tame foxes showed a significant increase in the *CYP26B1* mRNA as compared to aggressive animals ($p = 0.003$) (Fig. 3, *a*) and in *ADRA1A* as compared to both aggressive ($p = 0.027$) and unselected ($p = 0.038$) (see Fig. 3, *b*). Also, the *NR3C2* mRNA level in the ventral hippocampus of tame foxes was significantly higher than in unselected ones ($p = 0.011$) (Fig. 4).

Discussion

The presented data point to significant differences between the dorsal and ventral hippocampi in the levels of some mRNAs in foxes, as well as in other species: rats, mice, and humans (Cembrowski et al., 2016; Lee et al., 2017; Floriou-Servou et al., 2018; Vogel et al., 2020). Our results are by and large consistent with literature data on rats and mice (see Table 2). Recent studies of human gene expression (Allen Brain Atlas) demonstrate variation in the expression of about 5,000 genes along the hippocampus dorsoventral axis (Vogel et al., 2020). Some of these genes show linear or near-linear variation; thus, they can be regarded as axial position markers. Others demonstrate nonlinear expression profiles.

The *NR2F2* gene for transcription factor COUP-TFII is one of the markers most precisely indicating axial position. The amount of its mRNA in the ventral hippocampus is much greater than in the dorsal in all studies on rats, mice, and humans and in our study on foxes. Its expression in the adult hippocampus is confined mainly to GABAergic and glutamatergic neurons. The density of GABAergic neurons increases along the dorsoventral axis (Jinno, Kosaka, 2010), and so does *NR2F2* expression. Nevertheless, its expression in the dorsal hippocampus is confined to GABAergic neurons, which is indicative of their high density in the dorsal hippocampus as well (Fuentealba et al., 2010). The different functions of hippocampus compartments may be related to neuron distribution along the dorsoventral axis. It is known that the expression of acetylcholine receptor α_7 (*CHRNA7*) in the hippocampus, also confined to GABAergic neurons, is involved in aggression regulation (Lewis et al., 2018). However, we found no difference in *NR2F2* expression amongst the behavior groups; hence, aggression is controlled by other mechanisms.

Different species demonstrate inverse ratios between mRNA levels of some genes in the dorsal and ventral hippocampus compartments or no variation at all (see Table 2). For example, in our study such genes included *KCND3* and *NTS*. These differences can be explained by nonlinear expression profiles along the dorsoventral axis and putative sampling from nonidentical hippocampus sites in different experiments. In other cases, the species-specific expression of some genes may be related to morphological and functional features of the hippocampus itself. For instance, it is known that hippocampal neurogenesis in foxes considerably surpasses that in many mammals (Amrein, Slomianka, 2010). Morphological and functional features of various species may also stem from their ecology, in particular, spatial behavior in hoarding food (Jacobs et al., 1990; Jacobs, Spencer, 1994; Rehkämper et al., 2008; Amrein, 2015; Crosston et al., 2015; Lipp, 2017; Sonnenberg et al., 2019). It is presumable that the 15-fold hippocampal neurogenesis in red foxes as compared to dogs (Amrein, Slomianka, 2010) is related to this characteristic behavior (Sklepkovych, Montevecchi, 1996).

As the entire hippocampus and, especially, its ventral compartment, is the key region in stress response regulation, we investigated the glucocorticoid-mediated response of foxes at the present stage of selection: tame, aggressive, and unselected. The necessity of studying stress response at different stages of selection has been shown on another model, tame and aggressive Norway rats, which show no significant differences in the glucocorticoid-mediated response at the current stage (Prasolova et al., 2014). The detected significant differences amongst tame, aggressive, and unselected animals are consistent with earlier data (Trut et al., 2009). Thus, we can see that the restraint and combined restraint–emotional stresses induce similar differences in the cortisol-induced stress response in the experimental foxes, and these differences persist at the current stage of selection. However, the unselected animals showed an intermediate level of the glucocorticoid stress response between the tame and aggressive foxes. These discrepancies may be related to both the elevated stress response in the selection for aggressiveness and the unintentional selection of "unselected" foxes towards adaptation to coexistence with humans. Differences can also stem from different experiment designs (restraint stress with limited space in a shed *vs.* manual fixation) and measurement protocols (radioimmunoassay *vs.* HPLC).

It is likely that the weak response to different stress types in tame foxes is the main cause of high adult neurogenesis rate in the hippocampus, as shown in many studies on other species (Levone et al., 2015). Therefore, in search for molecular mechanisms modulating neurogenesis we first considered the levels of mRNAs for glucocorticoid (*NR3C1*, GR) and mineralocorticoid (*NR3C2*, MR) receptors

in the hippocampus. However, we found no differences in the levels of *NR3C1* mRNA amongst animals of different behavior genotypes in neither dorsal nor ventral hippocampus, although some research teams believed that these genes were important in domestication (Oskina et al., 2008; Pörtl, Jung, 2017). It is known that neuron progenitors in the subventricular zone express *NR3C1* but not *NR3C2* (Garcia et al., 2004). Apparently, *NR3C1* activation in these cells is the direct way by which glucocorticoids affect neurogenesis (Saaltink, Vreugdenhil, 2014). It is conceivable that *NR3C1* expression in this subpopulation of hippocampus cells varies amongst foxes differing in behavior.

In contrast, the ventral hippocampi of tame foxes contained more *NR3C2* mRNA than those of aggressive animals. It is likely that the mRNA level in the ventral hippocampi of unselected foxes is intermediate between tame and aggressive, but this difference is below the limits of qPCR accuracy. However, we may expect that in studies of individual splice variants (Three are known in rodents: α, β, and γ.) differences amongst groups in the expression of a particular splice variant will be more pronounced. It has been shown that their expression in cellular stress in a primary culture of cortex cells varies irregularly (Kang et al., 2009). Here we analyze only the total pool of *NR3C2* mRNA, because the fox genome had not been annotated in sufficient detail. It is known that NR3C2 levels in various types of hippocampus cells are different (Le Menuet, Lombès, 2014). Note that the difference was found just in the ventral hippocampus, whose contribution to stress response and emotion regulation is thought to be greater than that of the dorsal (Gulyaeva, 2019).

Studies on rodents demonstrate that elevated MR amount is associated with lower anxiety and active strategy of coping with stress. In females, it is also associated with weaker stress response (Lai et al., 2007; Rozeboom et al., 2007; Kanatsou et al., 2015; de Kloet et al., 2016). In addition, postmortem studies of humans show that depression lowers MR in the frontal (corresponding to rodent ventral) but not occipital (dorsal) hippocampus compartments, with no variation in GR (Medina et al., 2013). Changes in MR expression in rats under stress alter synaptic plasticity in the ventral but not dorsal hippocampus (Maggio, Segal, 2009; O'Leary, Cryan, 2014). It is likely that *NR3C2* expression in neuroglia can increase neurogenesis in the ventral hippocampus under acute stress (Le Menuet, Lombès, 2014; O'Leary, Cryan, 2014). Also, progenitor cell proliferation is lowered in mice with *NR3C2* knockout (Gass et al., 2000), whereas enhanced *NR3C2* expression in the forebrain accelerates progenitor proliferation and increases the population of young neurons in the dentate gyrus (Kanatsou et al., 2017). On the other hand, MR signaling in the hippocampus is involved in the regulation of the start and amplitude of stress response (Ratka et al., 1989; Harris et al., 2013; de Kloet et al., 2016), and stress initiation increases the MR level in the hippocampus (Veenema et al., 2003; de Kloet et al., 2016). The opposite effects of MR and their agonists and antagonists found in various studies may be related to different MR levels in animals at the start of the experiment (de Kloet et al., 2016). We conjecture that the detected high level of mRNA for MR (but not for GR) in the ventral hippocampus of tame foxes is one of the mechanisms that mitigate stress and anxiety in experimental domestication and, probably, indirectly enhance neurogenesis in this compartment. Further studies should be dedicated to the expression of splice variants of MR and their distribution in the hippocampus.

Foxes of different behavior genotypes differed in the contents of *CYP26B1* mRNA in the dorsal hippocampus and *ADRA1A* in the ventral one. As mentioned above, these contents varied along the dorsoventral axis of the hippocampus. The variation in mRNA contents found in the analysis of few samples from the dorsal hippocampi of tame and aggressive foxes by the RNAseq method (unpublished data) also points to the necessity of a comprehensive study of *CYP26B1* expression in hippocampus regions of the three fox populations. The *CYP26B1* gene encodes an enzyme of the cytochrome P450 superfamily. This enzyme catalyzes the degradation of all-trans retinoic acid (atRA), a vitamin A derivative. Changes in *CYP26B1* expression may be associated with different atRA concentrations in the hippocampi of tame and aggressive foxes.

It is known that atRA affects neurogenesis, but its effect looks graphically as an inverted U curve. All-trans retinoic acid deficiency reduces neuron differentiation, whereas higher concentrations enhance neurogenesis by stimulating both proliferation and differentiation of neural stem cells, and still higher atRA concentrations inhibit cell proliferation and affect the cognitive function and behavior (Kane et al., 2010; Hu et al., 2016, 2020; Stoney, McCaffery, 2016; Stoney et al., 2016; Mishra et al., 2018). High *CYP26B1* expression, apparently decreasing the atRA level, is associated with intense neurogenesis in the hippocampus of adult tame rats, earlier demonstrated by Huang et al. (2015). Our results seem to be consistent with the negative effect of atRA on neurogenesis in studies by P. McCaffery's (Stoney et al., 2016) and Zhou's (Hu et al., 2016, 2020) teams. It appears that *CYP26B1* inhibition reduces cell proliferation in the subgranular zone of the hippocampus in mice (Stoney et al., 2016). Probably, the atRA content in the hippocampus of tame foxes is close to the maximum level enhancing neurogenesis. The lower expression of *CYP26B1* in aggressive animals is associated with even higher atRA contents and, probably, lower neurogenesis. The unselected foxes, demonstrating lower neurogenesis than tame ones, seem to be in the middle between tame and aggressive. Note that genome-wide comparisons between village dogs and wolves and between humans and chimpanzees has also demonstrated differences in the atRA system (Theofanopoulou et al., 2017; Pendleton et al., 2018).

The effects of vitamin A and atRA on the HPA axis are complex and controversial. Dexamethasone upregulates the

expression of the *Aldh1a1* gene for an enzyme involved in atRA synthesis (Gil-Ibáñez et al., 2014). Chronic exposure to atRA and other retinoic acid forms enhances HPA axis activity and causes depression (Bremner, McCaffery, 2008; Cai et al., 2015). In particular, atRA induces overexpression of the *Crf*, *Crfr1*, and *Avp* genes in the hypothalamus (Cai et al., 2015). Probably, the lower HPA activity in tame foxes is partly related to the weakening of the atRA system. However, it should be noted that retinoic acid can, in contrast, lower the level of glucocorticoids, and, besides, it can exert an inverse effect on target tissues (Bonhomme et al., 2014; Hélène et al., 2016).

The detected high level of *ADRA1A* mRNA in the dorsal hippocampus of tame foxes is of special interest. This change may reflect the lower anxiety and elevated adult neurogenesis in the hippocampus as a result of lower HPA axis activity in the selection for tame behavior. Although the dorsal hippocampus contributes less to the regulation of stress responses and anxiety, its effects have been described in (Weaver et al., 2004; Gulyaeva, 2019). *ADRA1A* is presumed to play a role in the attention deficit disorder (Elia et al., 2009). The mechanisms mediating the effect of ADRA1A in behavior regulation are poorly understood, but it is known that long-term *ADRA1A* stimulation mitigates depression-like behavior and anxiety, and tricyclic antidepressants increase the ADRA1A receptor density in the forebrain of rodents (Deupree et al., 2007; Doze et al., 2009, 2011). Studies of the subependymal zone of lateral ventricles, which is another adult neurogenesis region along with the subgranular zone of the hippocampus, in transgenic mice demonstrate an association between high ADRA1A expression and high neurogenesis rate (Gupta et al., 2009). It is conceivable that ADRA1A also participates in hippocampal neurogenesis (Doze et al., 2011).

Conclusion

To sum up, we analyzed separately the dorsal and ventral hippocampi of foxes selected for contrasting behaviors and revealed differential expression of the *NR3C2*, *CYP26B1*, and *ADRA1A* genes, associated with both hippocampal neurogenesis and HPA axis regulation. Further studies of the expression of functional groups to which these genes belong are expected to shed light on hitherto unknown molecular mechanisms of domestication in general and on stress response weakening, elevated neurogenesis, and changes of the attitude to humans in domesticated animals in particular.

References

Amrein I. Adult hippocampal neurogenesis in natural populations of mammals. *Cold Spring Harb. Perspect. Biol.* 2015;7(5):a021295. DOI 10.1101/cshperspect.a021295.

Amrein I., Slomianka L. A morphologically distinct granule cell type in the dentate gyrus of the red fox correlates with adult hippocampal neurogenesis. *Brain Res.* 2010;1328:12-24. DOI 10.1016/j.brainres.2010.02.075.

Andersen C.L., Jensen J.L., Ørntoft T.F. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Res.* 2004;64:5245-5250. DOI 10.1158/0008-5472.CAN-04-0496.

Belyaev D.K. Destabilizing selection as a factor in domestication. *J. Hered.* 1979;70(5):301-308. DOI 10.1093/oxfordjournals.jhered.a109263.

Bonhomme D., Minni A.M., Alfos S., Roux P., Richard E., Higueret P., Moisan M.P., Pallet V., Touyarot K. Vitamin A status regulates glucocorticoid availability in Wistar rats: consequences on cognitive functions and hippocampal neurogenesis? *Front. Behav. Neurosci.* 2014;8:20. DOI 10.3389/fnbeh.2014.00020.

Bremner J.D., McCaffery P. The neurobiology of retinoic acid in affective disorders. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry.* 2008;32(2):315-331. DOI 10.1016/j.pnpbp.2007.07.001.

Cai L., Li R., Zhou J.N. Chronic all-trans retinoic acid administration induces CRF over-expression accompanied by AVP up-regulation and multiple CRF-controlling receptors disturbance in the hypothalamus of rats. *Brain Res.* 2015;1601:1-7. DOI 10.1016/j.brainres.2014.12.054.

Cembrowski M.S., Wang L., Sugino K., Shields B.C., Spruston N. Hipposeq: a comprehensive RNA-seq database of gene expression in hippocampal principal neurons. *eLife*. 2016;5:e14997. DOI 10.7554/eLife.14997.001.

Corradini B.R., Iamashita P., Tampellini E., Farfel J.M., Grinberg L.T., Moreira-Filho C.A. Complex network-driven view of genomic mechanisms underlying Parkinson's disease: analyses in dorsal motor vagal nucleus, locus coeruleus, and substantia nigra. *BioMed Res. Int*. 2014;2014:543673. DOI 10.1155/2014/543673.

Croston R., Branch C.L., Kozlovsky D.Y., Roth T.C., LaDage L.D., Freas C.A., Pravosudov V.V. Potential mechanisms driving population variation in spatial memory and the hippocampus in food-caching chickadees. *Integr. Comp. Biol*. 2015;55(3):354-371. DOI 10.1093/icb/icv029.

de Kloet E.R., Otte C., Kumsta R., Kok L., Hillegers M.H.J., Hasselmann H., Kliegel D., Joëls M. Stress and depression: a crucial role of the mineralocorticoid receptor. *J. Neuroendocrinol*. 2016;28(8). DOI 10.1111/jne.12379.

de Martino M.U., Alesci S., Chrousos G.P., Kino T. Interaction of the glucocorticoid receptor and the chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII): implications for the actions of glucocorticoids on glucose, lipoprotein, and xenobiotic metabolism. *Ann. N. Y. Acad. Sci.* 2004;1024:72-85. DOI 10.1196/annals.1321.006.

Deupree J.D., Reed A.L., Bylund D.B. Differential effects of the tricyclic antidepressant desipramine on the density of adrenergic receptors in juvenile and adult rats. *J. Pharmacol. Exp. Ther.* 2007;321(2):770-776. DOI 10.1124/jpet.106.118935.

Doze V.A., Handel E.M., Jensen K.A., Darsie B., Luger E.J., Haselton J.R., Talbot J.N., Rorabaugh B.R. α1A- and α1B-adrenergic receptors differentially modulate antidepressant-like behavior in the mouse. *Brain Res.* 2009;1285:148-157. DOI 10.1016/j.brainres.2009.06.035.

Doze V.A., Papay R.S., Goldenstein B.L., Gupta M.K., Collette K.M., Nelson B.W., Lyons M.J., Davis B.A., Luger E.J., Wood S.G., Haselton J.R., Simpson P.C., Perez D.M. Long-term α1A-adrenergic receptor stimulation improves synaptic plasticity, cognitive function, mood, and longevity. *Mol. Pharmacol.* 2011;80(4):747-758. DOI 10.1124/mol.111.073734.

Elia J., Capasso M., Zaheer Z., Lantieri F., Ambrosini P., Berrettini W., Devoto M., Hakonarson H. Candidate gene analysis in an on-going genome-wide association study of attention-deficit hyperactivity dis-

order: suggestive association signals in ADRA1A. *Psychiatr. Genet.* 2009;19(3):134-141. DOI 10.1097/YPG.0b013e32832a5043.

Floriou-Servou A., von Ziegler L., Stalder L., Sturman O., Privitera M., Rassi A., Cremonesi A., Thöny B., Bohacek J. Distinct proteomic, transcriptomic, and epigenetic stress responses in dorsal and ventral hippocampus. *Biol. Psychiatry.* 2018;84(7):531-541. DOI 10.1016/j.biopsych.2018.02.003.

Fuentealba P., Klausberger T., Karayannis T., Suen W.Y., Huck J., Tomioka R., Rockland K., Capogna M., Studer M., Morales M., Somogyi P. Expression of COUP-TFII nuclear receptor in restricted GABAergic neuronal populations in the adult rat hippocampus. *J. Neurosci*. 2010;30(5):1595-1609. DOI 10.1523/JNEUROSCI.4199-09.2010.

Garcia A., Steiner B., Kronenberg G., Bick-Sander A., Kempermann G. Age-dependent expression of glucocorticoid- and mineralocorticoid receptors on neural precursor cell populations in the adult murine hippocampus. *Aging Cell*. 2004;3(4):363-371. DOI 10.1111/j.1474-9728.2004.00130.x.

Gass P., Kretz O., Wolfer D.P., Berger S., Tronche F., Reichardt H.M., Kellendonk C., Lipp H.P., Schmid W., Schütz G. Genetic disruption of mineralocorticoid receptor leads to impaired neurogenesis and granule cell degeneration in the hippocampus of adult mice. *EMBO Rep.* 2000;1(5):447-451. DOI 10.1093/embo-reports/kvd088.

Gil-Ibáñez P., Bernal J., Morte B. Thyroid hormone regulation of gene expression in primary cerebrocortical cells: role of thyroid hormone receptor subtypes and interactions with retinoic acid and glucocorticoids. *PLoS One*. 2014;9(3):e91692. DOI 10.1371/journal.pone.0091692.

Gulyaeva N.V. Functional neurochemistry of the ventral and dorsal hippocampus: stress, depression, dementia and remote hippocampal damage. *Neurochem. Res.* 2019;44:1306-1322. DOI 10.1007/s11064-018-2662-0.

Gupta M.K., Papay R.S., Jurgens C.W.D., Gaivin R.J., Shi T., Doze V.A., Perez D.M. α1-Adrenergic receptors regulate neurogenesis and gliogenesis. *Mol. Pharmacol.* 2009;76(2):314-326. DOI 10.1124/mol.109.057307.

Haddjeri-Hopkins A., Tapia M., Ramirez-Franco J., Tell F., Marqueze-Pouey B., Amalric M., Goaillard J.M. Refining the identity and role of Kv4 channels in mouse substantia nigra dopaminergic neurons. *eNeuro*. 2021;8(4):ENEURO.0207-21.2021. DOI 10.1523/ENEURO.0207-21.2021.

Harris A.P., Holmes M.C., De Kloet E.R., Chapman K.E., Seckl J.R. Mineralocorticoid and glucocorticoid receptor balance in control of HPA axis and behaviour. *Psychoneuroendocrinol*. 2013;38(5):648-658. DOI 10.1016/j.psyneuen.2012.08.007.

Hélène R., Julie B., Aloïs L., Marie-Pierre M., Véronique P., Anabelle R., Jean-Benoît C. Retinoids and glucocorticoids have opposite effects on actin cytoskeleton rearrangement in hippocampal HT22 cells. *Int. J. Biochem. Cell Biol.* 2016;71:102-110. DOI 10.1016/j.biocel.2015.12.014.

Hu P., Wang Y., Liu J., Meng F.T., Qi X.R., Chen L., van Dam A.M., Joëls M., Lucassen P.J., Zhou J.N. Chronic retinoic acid treatment suppresses adult hippocampal neurogenesis, in close correlation with depressive-like behavior. *Hippocampus*. 2016;26(7):911-923. DOI 10.1002/hipo.22574.

Hu P., van Dam A.M., Wang Y., Lucassen P.J., Zhou J.N. Retinoic acid and depressive disorders: evidence and possible neurobiological mechanisms. *Neurosci. Biobehav. Rev.* 2020;112:376-391. DOI 10.1016/j.neubiorev.2020.02.013.

Huang S., Slomianka L., Farmer A.J., Kharlamova A.V., Gulevich R.G., Herbeck Yu.E., Trut L.N., Wolfer D.P., Amrein I. Selection for tameness, a key behavioral trait of domestication, increases adult hippocampal neurogenesis in foxes. *Hippocampus*. 2015;25(8):963-975. DOI 10.1002/hipo.22420.

Jacobs L.F., Gaulin S.J., Sherry D.F., Hoffman G.E. Evolution of spatial cognition: sex-specific patterns of spatial behavior predict hippocampal size. *Proc. Natl. Acad. Sci. USA*. 1990;87(16):6349-6352. DOI 10.1073/pnas.87.16.6349.

Jacobs L.F., Spencer W.D. Natural space-use patterns and hippocampal size in kangaroo rats. *Brain Behav. Evol*. 1994;44(3):125-132. DOI 10.1159/000113584.

Jinno S., Kosaka T. Stereological estimation of numerical densities of glutamatergic principal neurons in the mouse hippocampus. *Hippocampus.* 2010;20(7):829-840. DOI 10.1002/hipo.20685.

Kanatsou S., Fearey B.C., Kuil L.E., Lucassen P.J., Harris A.P., Seckl J.R., Krugers H., Joels M. Overexpression of mineralocorticoid receptors partially prevents chronic stress-induced reductions in hippocampal memory and structural plasticity. *PLoS One.* 2015;10(11):e0142012. DOI 10.1371/journal.pone.0142012.

Kanatsou S., Karst H., Kortesidou D., van den Akker R.A., den Blaauwen J., Harris A.P., Seckl J.R., Krugers H.J., Joels M. Overexpression of mineralocorticoid receptors in the mouse forebrain partly alleviates the effects of chronic early life stress on spatial memory, neurogenesis and synaptic function in the dentate gyrus. *Front. Cell. Neurosci*. 2017;11:132. DOI 10.3389/fncel.2017.00132.

Kane M.A., Folias A.E., Wang C., Napoli J.L. Ethanol elevates physiological *all-trans*-retinoic acid levels in select loci through altering retinoid metabolism in multiple loci: a potential mechanism of ethanol toxicity. *FASEB J.* 2010;24(3):823-832. DOI 10.1096/fj.09-141572.

Kang P., Rogalska J., Walker C.A., Burke M., Seckl J.R., Macleod M.R., Lai M. Injury-induced mineralocorticoid receptor expression involves differential promoter usage: a novel role for the rat MRβ variant. *Mol. Cell. Endocrinol.* 2009;305(1-2):56-62. DOI 10.1016/j.mce.2009.02.008.

Kukekova A.V., Johnson J.L., Xiang X., Feng S., Liu S., Rando H.M., Kharlamova A.V., Herbeck Yu., Serdyukova N.A., Xiong Z., Beklemischeva V., Koepfli K.-P., Gulevich R.G., Vladimirova A.V., Hekman J.P., Perelman P.L., Graphodatsky A.S., O'Brien S.J., Wang X., Clark A.G., Acland G.A., Trut L.N., Zhang G. Red fox genome assembly identifies genomic regions associated with tame and aggressive behaviours. *Nat. Ecol. Evol.* 2018;2(9):1479-1491. DOI 10.1038/s41559-018-0611-6.

Kvichansky A.A., Volobueva M.N., Manolova A.O., Bolshakov A.P., Gulyaeva N.V. Neonatal proinflammatory stress alters the expression of genes of corticosteroid receptors in the rat hippocampus: septo-temporal differences. *Neurochem. J.* 2017;11(3):255-258. DOI 10.1134/S1819712417030059.

Lai M., Horsburgh K., Bae S.E., Carter R.N., Stenvers D.J., Fowler J.H., Yau J.L., Gomez-Sanchez C.E., Holmes M.C., Kenyon C.J., Seckl J.R., Macleod M.R. Forebrain mineralocorticoid receptor overexpression enhances memory, reduces anxiety and attenuates neuronal loss in cerebral ischaemia. *Eur. J. Neurosci.* 2007;25(6):1832-1842. DOI 10.1111/j.1460-9568.2007.05427.x.

Le Menuet D., Lombès M. The neuronal mineralocorticoid receptor: from cell survival to neurogenesis. *Steroids*. 2014;91:11-19. DOI 10.1016/j.steroids.2014.05.018.

Lee A.-R., Kim J.-H., Cho E., Kim M., Park M. Dorsal and ventral hippocampus differentiate in functional pathways and differentially associate with neurological disease-related genes during postnatal development. *Front. Mol. Neurosci.* 2017;10:331. DOI 10.3389/fnmol.2017.00331.

Levone B.R., Cryan J.F., O'Leary O.F. Role of adult hippocampal neurogenesis in stress resilience. *Neurobiol. Stress*. 2015;1:147-155. DOI 10.1016/j.ynstr.2014.11.003.

Lewis A.S., Pittenger S.T., Mineur Y.S., Stout D., Smith P.H., Picciotto M.R. Bidirectional regulation of aggression in mice by hippocampal alpha-7 nicotinic acetylcholine receptors. *Neuropsychopharmacology*. 2018;43(6):1267-1275. DOI 10.1038/npp.2017.276.

Lipp H.P. Evolutionary shaping of adult hippocampal neurogenesis in mammals-cognitive gain or developmental priming of personality traits? *Front. Neurosci.* 2017;11:420. DOI 10.3389/fnins.2017.00420.

Livak K.J., Schmittgen T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods*. 2001;25(4):402-408. DOI 10.1006/meth.2001.1262.

Lowe A., Dalton M., Sidhu K., Sachdev P., Reynolds B., Valenzuela M. Neurogenesis and precursor cell differences in the dorsal and ventral adult canine hippocampus. *Neurosci. Lett*. 2015;593:107-113. DOI 10.1016/j.neulet.2015.03.017.

Maggio N., Segal M. Differential modulation of long-term depression by acute stress in the rat dorsal and ventral hippocampus. *J. Neurosci.* 2009;29(27):8633-8638. DOI 10.1523/JNEUROSCI.1901-09.2009.

Medina A., Seasholtz A.F., Sharma V., Burke S., Bunney W., Jr., Myers R.M., Schatzberg A., Akil H., Watson S.J. Glucocorticoid and mineralocorticoid receptor expression in the human hippocampus in major depressive disorder. *J. Psychiatr. Research.* 2013;47(3):307-314. DOI 10.1016/j.jpsychires.2012.11.002.

Ming G., Song H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron*. 2011;70(4):687-702. DOI 10.1016/j.neuron.2011.05.001.

Mishra S., Kelly K.K., Rumian N.L., Siegenthaler J.A. Retinoic acid is required for neural stem and progenitor cell proliferation in the adult hippocampus. *Stem Cell Rep*. 2018;10(6):1705-1720. DOI 10.1016/j.stemcr.2018.04.024.

O'Leary O.F., Cryan J.F. A ventral view on antidepressant action: roles for adult hippocampal neurogenesis along the dorsoventral axis. *Trends Pharmacol. Sci.* 2014;35(12):675-687. DOI 10.1016/j.tips.2014.09.011.

O'Rourke T., Boeckx C. Glutamate receptors in domestication and modern human evolution. *Neurosci. Biobehav. Rev.* 2020;108:341-357. DOI 10.1016/j.neubiorev.2019.10.004.

Oskina I.N., Herbeck Yu.E., Shikhevich S.G., Plyusnina I.Z., Gulevich R.G. Alterations in the hypothalamus-pituitary-adrenal and immune systems during selection of animals for tame behavior. *Informatsionnyy Vestnik VOGiS = The Herald of Vavilov Society for Geneticists and Breeders*. 2008;12(1/2):39-49. (in Russian)

Ovchinnikov V.Yu., Antonov E.V., Vasilyev G.V., Shihevich S.G., Shepeleva D.V., Herbeck Yu.E. Hippocampal glucocorticoid receptor and microRNA gene expression and serum cortisol concentration in foxes selected for behavior toward humans. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding*. 2018;22(2):230-234. DOI 10.18699/VJ18.352. (in Russian)

Pendleton A.L., Shen F., Taravella A.M., Emery S., Veeramah K.R., Boyko A.R., Kidd J.M. Comparison of village dog and wolf genomes highlights the role of the neural crest in dog domestication. *BMC Biol*. 2018;16(1):e64. DOI 10.1186/s12915-018-0535-2.

Pörtl D., Jung C. Is dog domestication due to epigenetic modulation in brain? *Dog Behav*. 2017;3(2):21-32. DOI 10.4454/db.v3i2.55.

Prasolova L.A., Gerbek Yu.E., Gulevich R.G., Shikhevich S.G., Konoshenko M.Y., Kozhemyakina R.V., Oskina I.N., Plyusnina I.Z. The effects of prolonged selection for behavior on the stress response and activity of the reproductive system of male grey rats (*Rattus norvegicus*). *Russ. J. Genet.* 2014;50(8):846-852. DOI 10.1134/S1022795414080031.

Price E.O. Animal Domestication and Behavior. Oxford; New York: Oxford Univ. Press, 2002.

Ratka A., Sutanto W., Bloemers M., de Kloet E.R. On the role of brain mineralocorticoid (type I) and glucocorticoid (type II) receptors in neuroendocrine regulation. *Neuroendocrinology.* 1989;50(2):117-123. DOI 10.1159/000125210.

Rehkämper G., Frahm H.D., Cnotka J. Mosaic evolution and adaptive brain component alteration under domestication seen on the background of evolutionary theory. *Brain Behav. Evol*. 2008;71(2):115-126. DOI 10.1159/000111458.

Rozeboom A.M., Akil H., Seasholtz A.F. Mineralocorticoid receptor overexpression in forebrain decreases anxiety-like behavior and alters the stress response in mice. *Proc. Natl. Acad. Sci. USA*. 2007;104(11):4688-4693. DOI 10.1073/pnas.0606067104.

Saaltink D.J., Vreugdenhil E. Stress, glucocorticoid receptors, and adult neurogenesis: a balance between excitation and inhibition? *Cell. Mol. Life Sci.* 2014;71:2499-2515. DOI 10.1007/s00018-014-1568-5.

Sklepkovych B.O., Montevecchi W.A. Food availability and food hoarding behaviour by red and arctic foxes. *Arctic*. 1996;49(3):228-234. DOI 10.14430/arctic1199.

Sonnenberg B.R., Branch C.L., Pitera A.M., Bridge E., Pravosudov V.V. Natural selection and spatial cognition in wild food-caching mountain chickadees. *Curr. Biol*. 2019;29(4):670-676. DOI 10.1016/j.cub.2019.01.006.

Stoney P.N., McCaffery P. A vitamin on the mind: new discoveries on control of the brain by vitamin A. *World Rev. Nutr. Diet.* 2016;115:98-108. DOI 10.1159/000442076.

Stoney P.N., Fragoso Y.D., Saeed R.B., Ashton A., Goodman T., Simons C., Gomaa M.S., Sementilli A., Sementilli L., Ross A.W., Morgan P.J., McCaffery P.J. Expression of the retinoic acid catabolic enzyme CYP26B1 in the human brain to maintain signaling homeostasis. *Brain Struct. Funct.* 2016;221:3315-3326. DOI 10.1007/s00429-015-1102-z.

Theofanopoulou C., Gastaldon S., O'Rourke T., Samuels B.D., Messner A., Martins P.T., Delogu F., Alamri S., Boeckx C. Self-domestication in *Homo sapiens*: insights from comparative genomics. *PloS One*. 2017;12(10):e0185306. DOI 10.1371/journal.pone.0185306.

Todorov O.S., Weisbecker V., Gilissen E., Zilles K., De Sousa A.A. Primate hippocampus size and organization are predicted by sociality but not diet. *Proc. Royal Soc. B.* 2019;286(1914):20191712. DOI 10.1098/rspb.2019.1712.

Trut L.N., Plyusnina I.Z., Oskina I.N. An experiment on fox domestication and debatable issues of evolution of the dog. *Russ. J. Genet.* 2004;40(6):644-655. DOI 10.1023/B:RUGE.0000033312.92773.c1.

Trut L., Oskina I., Kharlamova A. Animal evolution during domestication: the domesticated fox as a model. *BioEssays*. 2009;31(3):349-360. DOI 10.1002/bies.200800070.

Trut L.N., Kharlamova A.V., Pilipenko A.S., Herbeck Yu.E. The fox domestication experiment and dog evolution: A view based on modern molecular, genetic, and archaeological data. *Russ. J. Genet.* 2021;57(7):778-794. DOI 10.1134/S1022795421070140.

Truvé K., Parris T.Z., Vizlin-Hodzic D., Salmela S., Berger E., Ågren H., Funa K. Identification of candidate genetic variants and altered protein expression in neural stem and mature neural cells support altered microtubule function to be an essential component in bipolar disorder. *Transl. Psychiatry*. 2020;10(1):e390. DOI 10.1038/s41398-020-01056-1.

Tzakis N., Holahan M.R. Social memory and the role of the hippocampal CA2 region. *Front. Behav. Neurosci*. 2019;13:233. DOI 10.3389/fnbeh.2019.00233.

Veenema A.H., Neumann I.D. Neurobiological mechanisms of aggression and stress coping: a comparative study in mouse and rat selection lines. *Brain Behav. Evol.* 2007;70(4):274-285. DOI 10.1159/000105491.

Veenema A.H., Meijer O.C., de Kloet E.R., Koolhaas J.M. Genetic selection for coping style predicts stressor susceptibility. *J. Neuroendocrinol.* 2003;15:256-267. DOI 10.1046/j.1365-2826.2003.00986.x.

Vogel J.W., La Joie R., Grothe M.J., Diaz-Papkovich A., Doyle A., Vachon-Presseau E., Lepage C., Vos de Wael R., Thomas R.A., Iturria-Medina Y., Bernhardt B., Rabinovici G.D., Evans A.C. A molecular gradient along the longitudinal axis of the human hippocampus informs large-scale behavioral systems. *Nat. Commun.* 2020;11(1):960. DOI 10.1038/s41467-020-14518-3.

Weaver I.C.G., Cervoni N., Champagne F.A., D'Alessio A.C., Sharma S., Seckl J.R., Dymov S., Szyf M., Meaney M.J. Epigenetic programming by maternal behavior. *Nat. Neurosci.* 2004;7:847-854. DOI 10.1038/nn1276.

Xiao F., Zhang X., Ni P., Yu H., Gao Q., Li M., Huo P., Wei Z., Wang S., Zhang Y., Zhao R., Li A., Li Z., Li Yu., Cheng H., Du L., Ren S., Yu Q., Liu Ya., Zhao Yu. Voltage-dependent potassium channel Kv4.2 alleviates the ischemic stroke impairments through activating neurogenesis. *Neurochem. Int*. 2021;50:e105155. DOI 10.1016/j.neuint.2021.105155.

Ye J., Coulouris G., Zaretskaya I., Cutcutache I., Rozen S., Madden T.L. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinform.* 2012;13:134. DOI 10.1186/1471-2105-13-134.

Acknowledgements. This work was supported by the Russian Science Foundation, project 21-44-04405. The breeding of experimental animals in the Shared Access Center for Gene Pools of Fur and Farm Animals, Institute of Cytology and Genetics, was supported by State Budgeted Project FWNR-2022-0019.

The authors are grateful to V.V. Ivaykin, A.V. Vladimirova, I.V. Pivovarova, T.I. Semenova, V.I. Vladimirova, T.V. Konovalova, and all the staff of the Shared Access Center for assistance in the study. They also acknowledge the significant contribution of the reviewers to manuscript improvement.

Conflict of interest. The authors declare no conflict of interest.

Received December 1, 2022. Revised June 18, 2023. Accepted June 30, 2023.