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ANIMAL AND HUMAN PHYSIOLOGY

The Effect of Photoperiod Duration on Humoral Innate and Humoral Adaptive Immune Responsiveness in Campbell's Hamster

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Abstract—We studied the humoral innate immune responsiveness (HII), i.e., the hemolysis of rabbit erythrocytes by complement proteins, the adaptive humoral (antibody production) immune responsiveness (AHI) to intraperitoneal injection of sheep red blood cells, the morphological and hormonal reproductive characteristics and stress level (blood cortisol) in male Campbell's hamsters kept under long-day (LD; 16D: 8N) and short-day (SD; 8D: 16N) photoperiods. In SD males we found the body mass, anogenital distance, midventral gland size, and level of testosterone in the peripheral blood (but not the cortisol level) decreased after two months of exposure. The results indicate lower HII, but not AHI in SD. Comparison of the SD nonresponders, SD responders, and LD individuals demonstrated a statistically significant increase of HII in SD photosensitive hamsters compared to LD. There was no link between HII and AHI, which indicates an independent photoperiodic responsiveness of different branches of the immune system.

Keywords: photoperiodism, constitutive innate immunity, adaptive immunity, winter immunity enhancement, *Phodopus campbelli*

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INTRODUCTION

The well-known phenomenon of winter immunity enhancement in seasonally breeding mammals in temperate and cold climatic zones (Sinclair and Lochmiller, 2000) can be considered as an adaptation to counteract seasonal infections, which are more common in autumn and winter (Nelson, 2004). The mechanism of winter immunity enhancement is usually explained from the standpoint of two hypotheses that do not exclude one another—the hypothesis of an endogenous bolstering mechanism that enhances immune function in winter under the action of winter stressors (winter food shortage, low temperatures) (Nelson and Demas, 1996; Sinclair and Lochmiller, 2000; Nelson, 2004) and the tradeoff hypothesis, according to which winter immunity enhancement occurs as a result of a decrease in reproductive effort accompanying resource-costly functions (Martin et al., 2004, 2006; Greenman et al., 2005). Indeed, it is well known that the length of daylight is a signaling factor in seasonally breeding mammals that determines a number of functions, primarily reproductive ones (see the review Prendergast et al., 2002; Hazlerigg, 2012). With regard to immunity, the photoperiod effects are not so unambiguous, and the research results are contradictory (Martin et al., 2008; Ter Horst et al., 2021). In nature, the effect of immunity enhancement can be masked in winter by the action of winter stressors and in summer by internal stress due to reproduction, molting, adaptation to forcoming winter (Martin et al., 2008). Differences in lifestyle and preferred habitats may also explain winter immunity enhancement in some species and its absence in others (Nelson, 2004; Lee, 2006; Martin et al., 2008).

The works devoted to the study of seasonal variability of immunity usually concern one branch, less often two, of a multi-layered immune system (Martin et al., 2008). The question of the extent to which different branches of immunity undergo seasonal variation and how much the depth of such changes can vary in different species remains controversial (Lohmiller and Moshkin, 1999; Martin et al., 2008; Scheiermann et al., 2018; Onishi et al., 2020). The direction of seasonal effect may depend on which branch of immune system (innate or adaptive, cell-mediated or humoral immunity) is being studied and on the methods used (Martin et al., 2008; Zysling et al., 2009; Adelman et al., 2013; Stevenson and Prendergast, 2015; Schults et al., 2017).

The simplest way to compare the response of different branches of the immune system to seasonal factors is a laboratory experiment based on change in daylight exposure with standardization of all other animal care conditions (Nelson, 2004). Undoubtedly, the species specific features of the winter biology can affect the results of experiments. Therefore, to resolve

controversial issues related to seasonal changes in immunity, it seems promising to study taxonomically close species that differ in adaptations for winter. Hamsters of the genus *Phodopus* are a convenient model for this kind of research.

The Djungarian hamster (*Phodopus sungorus* Pallas, 1773) has been used in chronobiological studies since the middle of the last century. Under short-day conditions, males experience a decrease in reproduction with a simultaneous increase in aggressiveness (Jasnow et al., 2000; Scotti et al., 2007; Bedrosian et al., 2012; Rendon et al., 2016) and an increase in the blood concentration of glucocorticoids (Bilbo et al., 2002). The hamsters experience a suppression of the secondary antibody production in response to the antigen KLH (Prendergast et al., 2004). At the same time, on a short day, different indicators of innate immunity change in different directions. Under the short day-light regime males of Djungarian hamster show an increase in the cutaneous DNFB-induced response, and in the absolute number of blood leukocytes and lymphocytes (Bilbo et al., 2002; Prendergast et al., 2004). Also, there was a decrease in the severity of the sickness behavior syndrome, reflecting the effect on the brain of cytokines released by T-lymphocytes in response to different evolutionary distinct antigens (Baillie and Prendergast, 2008). Peritonitis did not develop in response to intraperitoneal administration of zymosan (Pawlak et al., 2009). These data indicate the presence of a complex relationship between various physiological processes involved in the regulation of innate immunity and triggered by of the day length shortage (Pawlak et al., 2009).

The results of experiments on the effect of a short day on the immunity of desert hamster (*Phodopus roborovskii* Satunin, 1903) are also contradictory. In males, the short-day caused involution of reproductive organs, a decrease in body mass, an increase in the mass-specific basal metabolic rate, in mass-specific maximum metabolic rate, and in the background level of stress, but did not affect the adaptive humoral immune response to sheep red blood cells. At the same time, the number of granulocytes in the peripheral blood decreased, but the number of lymphocytes increased (Vasilieva et al., 2020).

There are no data on the effect of daylight duration on the immunity characteristics of the third species of the genus, Campbell's hamster (*Phodopus campbelli* Thomas, 1905). It inhabits dry steppes and semi-deserts of Central Asia and It is known as a seasonal breeder (Sokolov and Orlov, 1980; Rogovin et al., 2014a). It has been shown that immature individuals of different laboratory populations, which originated from different parts of the species range differ in their response to the short day regime. The pronounced inhibition of growth and maturation was demonstrated by descendants of animals which came from the extreme western part of the range, the Chuya steppe (Vasilieva and Parfenova, 2003), and which, according to mtDNA analysis, represent an independent clade (Meshchersky and Feoktistova, 2009). Therefore, hamsters of this line were chosen as a model for testing the assumption that the suppression of the reproduction induced by a short day can lead to an increase in the immunocompetence of animals.

In this study, we attempted to evaluate (1) the effect of the short-day regime on innate and adaptive immunoresponsiveness and the reproductive and morphological characteristics of hamsters, (2) the relationship between target indicators of the activity of innate and adaptive immunity, and (3) the relationship of each of the immune indicators with the reproductive condition of males kept on short or long days.

MATERIALS AND METHODS

Animals

The experimental hamsters originated from the laboratory population of the Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, which descended from individuals captured in the 1980s–1990s from the population of Kosh-Agach (Kosh-Agach district of the Altai Republic). The animals were kept under standardized conditions, with a photoperiod of 16D : 8N and a temperature of 22 ± 2°C. Food (rodent chow for rats, mice, and hamsters, oats, sunflower seeds, brown bread, fresh vegetables) and water were provided *ad libitum*. Wood shavings were used as bedding. The bedding was changed every ten days, with at least a week before any manipulations with animals. All cages were equipped with shelters and running wheels.

Design of Experiment

The experimental males stayed with their parents and siblings until the age of one month. Then they were kept in plastic cages (70 × 40 × 40 cm) in same-sex groups of ten individuals. On July 1, 2021, all hamsters of 2–3 months age were weighed. Their body length , anogenital distance (AGD), and the size of the midventral gland (MVG) ($L \times l$) were measured. MVG reflects the reproductive status of males (Sokolov et al., 1990). Then these animals were divided into small groups of 4–5 individuals, in such a way that the groups' composition was aligned according to the specified morphometric parameters and age. After 25 days, pairs of groups selected in this way were equally divided into experimental groups (short day, SD, 6D : 18N) (37 males) and control groups, which continued to be kept on a long day (LD, 16D : 8N) (40 males). Lights in both rooms were turned on at 8 a.m. At the beginning of the experiment, there were no differences between the SD and LD samples in terms of the morphometric parameters and age.

Two months later, between September 24 and 27, 2021, blood samples were taken from the males to determine the serum testosterone and cortisol levels and to characterize the activity of humoral innate immunity (see below for more information).

Blood (about 350 μL) was taken from the sublingual venous sinus according to the modified method of B.M. Graievskaya (Graievskaya et al., 1986) at the same time at the beginning of the light phase (within 1–2 hours after turning on the light). The procedure for taking a sample from one animal took no more than 1.5 min, which is two times less than the time for the release of glucocorticoids into the blood in response to stress (Rogovin et al., 2014b). The serum was centrifuged for 15 min with RCF = 800, frozen and stored at –20°C until analysis.

After taking the blood, the hamsters were weighed with an accuracy of 0.1 g, their body length, AGD, and length and width of the MVG were measured with an accuracy of 0.1 mm with a digital caliper.

Immunization of the hamsters with sheep red blood cells from defibrinated blood preserved in Olsver solution (LLC KrolInfo, Orekhovo-Zuyevo, Moscow oblast, Russia) was carried out on October 11–12, 2021, and October 13–14, 2021. In the interval from 18 to 19 p.m. males were injected intraperitoneally with 2 μL/g suspension of sheep red blood cells (SRBC) at a volume concentration of 2% in a physiological solution. Preliminarily, SRBC were washed three times with physiological saline to remove the preserving agent by centrifugation at RCF = 800 for 15 min. The resulting erythrocyte mass was suspended in saline to the required concentration. On the 7th day after immunization, a second portion of blood was taken from the sublingual vein to assess the titer of antibodies to SRBC at the peak of the immune response (Rogovin et al., 2014b). After blood sampling, the hamsters were weighed, their body length, anogenital distance, and length and width of the midventral gland were measured.

Innate Immunoresponsiveness

The hemolysis-hemagglutination assay of erythrocytes was first proposed to characterize the state of the constitutional innate immunity in different bird species by K. Matson et al. (2005). Since then, the method has been applied repeatedly by ornithologists with no modifications (Butler et al., 2013). In studies of mammals, the method of K. Matson et al. was used less frequently and with modifications, which were associated with the physiological and biochemical differences between the mammalian species and birds studied (Gilot-Fromont et al., 2012; Racca et al., 2014; Heinrich et al., 2017; Ruoss et al., 2019; etc.).

In this study, we used the method of K. Matson et al. (2005), which was partially modified by us. We estimated exclusively the hemolysis of alien erythrocytes with the hamster blood serum. We declined to use heparin when taking blood due to the weak severity of the hemagglutination reaction overlapped by lysis. The addition of erythrocytes as an antigen to the blood serum of nonimmunized hamsters gives a well-defined picture of hemolytic activity, which also may be associated with a complement activation cascade along an alternative pathway without the participation of antibodies.

Rabbit erythrocytes (RRBC) were chosen as alien red blood cells (Seto and Henderson, 1968; Matson et al., 2005), because a preliminary test showed that their use gave higher levels of hemolytic activity compared to the SRBC traditionally used in studies of adaptive humoral immunoresponsiveness (Wilcoxon Matched Pairs Test; $Z = 2.49$; $N_1 = N_2 = 11$; $p = 0.01$).

RRBC were obtained from defibrinated blood preserved in Olsver solution (1 : 1; LLC KrolInfo, Orekhovo-Zuyevo, Moscow oblast, Russia). Erythrocytes were washed four times with saline solution with suspension centrifugation in a gentle mode (RCF = 200) for 5 min. The working volume concentration of the erythrocyte suspension was calculated using hematocrit capillaries. Physiological saline was used instead of the recommended 0.01 M phosphate buffered saline (Matson et al., 2005) because it provided better resolution for visual assessment of hemolysis. Three concentrations of erythrocyte suspension added to the hamsters blood serum were preliminarily tested: 0.5% (Rogovin et al., 2014b), 1% (Matson et al., 2005), and 0.75%. When assessing the hemolytic activity of the serum, the sensitivity of the method turned out to be higher when using a 0.5% suspension of RRBC in saline compared to the recommended 1% suspension (Wilcoxon Matched Pairs Test: $Z = 2.2$, $p = 0.028$, $N_1 = N_2 = 10$). Given the low normal hemolysis rates, a concentration of 0.5% may be preferred, because it makes the method more sensitive. On the other hand, 1% RRBC suspension gives better reproducibility of the result and a clearer assessment of the reaction boundary, simplifying the visual assessment at the expense of the sensitivity of the method (Fig. 1). We settled on an intermediate option—a volume concentration of 0.75%. This concentration also ensures good reproducibility of the estimate ($y = 0.97x - 0.11$; $R^2 = 0.93$; $n = 14$).

A 96-well polystyrene U-shaped immunoassay plate was used. Using an automatic dispensing pipette, 25 μL of hamster blood serum was added to wells 1 and 2, and 25 μL of physiological saline was added to wells 2–9. The titration was started from well 2, transferring 25 μL of the mixed content from well to well. From well 8, 25 μL of liquid was removed after stirring. Well 9 served as a negative control. 25 μL of the suspension of rabbit erythrocytes in physiological saline at a volume concentration of 0.75% was added to wells 1–9. The plate was covered with a lid after light stirring, placed in a plastic bag, and incubated at a temperature

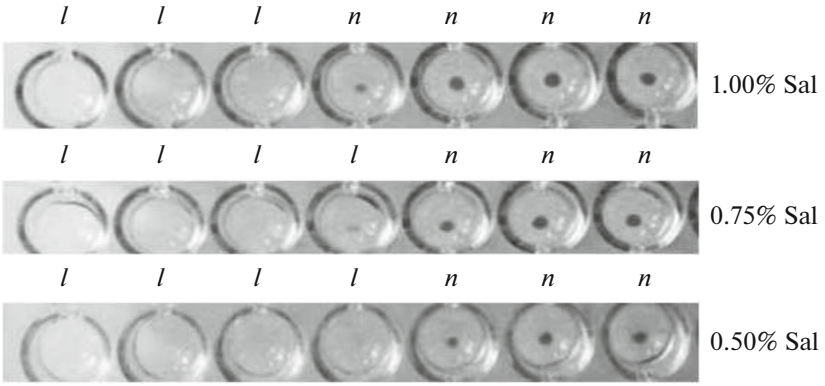

Fig. 1. An example of the reaction of hemolysis of rabbit erythrocytes with the blood serum of male Campbell's dwarf hamsters. Digital photo. Sal is the physiological solution; *l* is lysis, and *n* means no reaction.

of 38°C for 90 minutes. The temperature regime was selected as optimal for the species earlier when assessing hemagglutination after immunization of Campbell's dwarf hamsters with SRBC (Rogovin et al., 2014b). After 90 min, the plate was removed and left at room temperature to visualize the maximum hemolytic reaction for 20 min in an inclined position at an angle of 45° and then for another 70 min in the horizontal position (Matson et al., 2005). The criterion for assessing the intensity of hemolysis of the introduced erythrocytes was the number of the well, after which the reaction was no longer observed (Fig. 1).

Adaptive Humoral (B-Cell) Immunoresponsiveness

The serum level of antibodies in the immunized hamsters was determined by the hemagglutination reaction (Wegmann and Smithies, 1966) in the wells of a 96-well U-shaped immunological plate by titrating the blood serum samples in the wells and adding a 0.5% suspension of SRBC (LLC KrolInfo, Orekhovo-Zuyevo, Moscow oblast, Russia) in saline to the serum samples in multiple dilutions. Erythrocytes from defibrinated sheep blood preserved with Olsver solution were beforehand washed three times from Olsver with centrifugation at RCF = 800. Taking into account the normally well-pronounced humoral immune response induced by immunization, introduction into the wells began from the 3rd well of the row in order to save the hamster blood serum. Preliminarily, 50 μL of physiological saline was added to the wells, starting from the 4th one. Then 25 μL of the serum sample and 75 μL of the physiological saline were added to the third well and thoroughly mixed with a dispensing pipette, and 50 μL of the suspension was transferred to the next well, thoroughly mixed, etc. Next, 50 μL of the content was removed from the last well of the row. Finally 50 μL of the erythrocyte suspension was added to the wells. The sealed plate was incubated for 120 min at 38°C. The serum antibody titer (ABT) was visually assessed by the number of the last well of the plate, which, with successive multiple dilutions, contained an amount of antibodies that was still sufficient for agglutination. The serial number of the well was used as an indicator of the intensity of the immune response. In the case of a value intermediate between two adjacent wells, 0.5 was added to the number of the previous well. In rare cases, when there was no reaction in the 3rd well, an assessment was made for high serum concentrations: the first well, 50 μL of serum; the second well (from which the titration started), 50 μL of serum + 50 μL of saline.

Hormones

Serum testosterone and cortisol concentrations were assessed by enzyme-linked immunosorbent assay (ELISA). The ready-made test systems ELISA-TS (testosterone) and ELISA-cortisol (CJSC NVO Immunotech, Moscow, Russia) were used. The cross-reaction of cortisol with testosterone for these sets was 0.08%. The optical density was measured on a Multiskan FC ThermoScientific plate reader at a wavelength of 450 nm. Hormone concentrations were calculated automatically immediately after measurement using the Scanlt Software 6.0.1. If the testosterone concentration turned out to be higher than the sensitivity limit of the test system proposed by the manufacturer, the serum was diluted with the buffer "Solution for diluting blood serum" from the same manufacturer.

Statistics

Statistical analysis was carried out using the STATISTICA 10.0 software package (StatSoft Inc., United States). Comparisons of independent samples of males after being kept on the SD and LD regimens were carried out for the following variables: (1) innate immunity (hemolysis, c.u.), (2) acquired B-cell immunity (c.u.), (3) background level of testosterone (before immunization with SRBC; Lg, nmol/L), (4) background level of cortisol (before immunization

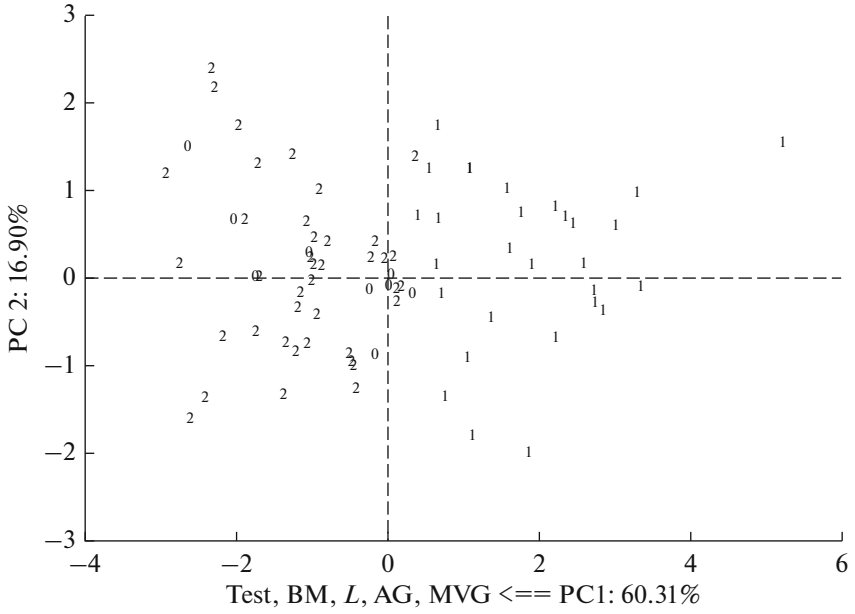

Fig. 2. The position of individual samples of SD nonresponders (0), SD responders (1) and LD (2) hamsters in the space of two principal components (PC1 and PC2) according to the results of the analysis of the interrelated reproductive characteristics in males after being on a short day (SD) or a long (day LD): Test is the background testosterone level, BM is body mass, *L* is body length, AG is anogenital distance, and MVG is midventral gland. The value increases from right to left. PC1 explains 60.31% of the total variance; PC2 explains 16.9% of the variance and is poorly interpreted.

with SRBC; Lg, nmol/L), (5) body mass (g), (6) body length (mm), (7) ratio of body mass to body length, (8) anogenital distance, AGD (mm), (9) midventral gland, MVG (mm^2).

To assess the deviations from normal distributions, the Shapiro–Wilk test was used as the main test, and, in parallel with the visual assessment of histograms, the Kolmogorov–Smirnov test was used as an additional test. To assess the homogeneity of the distributions, Levene's test for homogeneity of variances was used. The distributions were normalized taking decimal logarithms of the values of hormone concentrations. To compare the normally distributed characteristics of SD and LD males, means with the standard error and Student's *t*-test for independent samples were used. In cases of significant deviations from the normal distribution according to the Shapiro–Wilk test, the nonparametric Mann–Whitney *U*-test was used. Table 1 shows the values of the criteria for assessing differences by parametric and nonparametric methods for the variables the distributions of which satisfied the Kolmogorov–Smirnov test, but did not satisfy the Shapiro–Wilk test. The means and medians with limits of variation are given in Table 1 for all cases. The relationship between individual parameters of innate and adaptive immunity in hamsters in the SD and LD groups was assessed using the nonparametric Spearman rank correlation coefficient, since the distributions according to the Shapiro–Wilk test differed from normal.

Since the body weight and length, testosterone level, AGD, and MVG size turned out to be interrelated characteristics, we used the Principal Component Analysis (PCA) to combine these variables into one integral variable that generally characterizes an activity of reproductive system. The data were standardized. Since the first component explained 60.3% of the total variance, and the factor loadings of all variables included in the analysis ranged from –0.86 (ADG) to –0.63 (MVG), we used this integral variable to separate short-day nonresponders in the SD group from SD responders. This was done by comparing the distributions along the integral factorial axis PC1 of the obtained values in the SD and LD groups. The second component explained only 16.9% of the variance and was poorly interpretable (Fig. 2). The animals in which the individual values of the integral indicator were outside the distribution area of individual values in the LD group were considered SD responders. One-way analysis of variance (one-way ANOVA) was used to assess the effect of the photoperiod on innate and adaptive humoral immunoresponsiveness, and Tukey's test (HSD test) was used for subsequent paired comparisons.

RESULTS

Males kept on a short day (SD) had significantly lower mean body mass and length, body mass-to-length ratio, AGD, MVG size, and blood testosterone level than those kept on a long day (LD). At the same

Table 1. Morphophysiological characteristics of male Campbell's dwarf hamsters kept under short-day (SD) and long-day (LD) conditions. Short-day nonresponders in the SD group are included in the analysis (full sample)

Characteristic	Short day (SD)			Long day (LD)			t_{St}	p	$Z_{MW\text{-}U}$	p
	mean ± SE†	median (min–max)‡	N	mean ± SE	median (min–max)	N				
Innate immunity	2.69 ± 0.23	2.5 (0–7)	37	1.80 ± 0.19	2 (0–5)	40	2.98	**0.004**	2.851	**0.004**
Adaptive immunity	6.32 ± 0.27	6 (1.5–9)	37	6.42 ± 0.35	6.25(0–10)	40	–0.22	0.82	–0.67	0.50
Testosterone (Lg, nm/L)	0.12 ± 0.11	0.11 (–3.0–1.34)	37	0.83 ± 0.06	0.83 (0.21–1.48)	40	–5.72	**<0.001**	–5.41	**<0.001**
Cortisol (Lg, nm/L)	1.54 ± 0.05	1.51 (0.86–2.27)	37	1.63 ± 0.06	1.60 (1.06–2.43)	40	–0.95	0.34		
Body weight, g	45.07 ± 1.55	45 (32.0–69.5)	37	56.88 ± 0.95	56.7 (44.5–69.0)	40	–6.62	**<0.001**		
Body length, mm	97.3 ± 0.9	98 (88–114)	37	102.7 ± 0.8	103 (95–115)	40	–4.39	**<0.001**		
Weight/length	0.46 ± 0.01	0.45 (0.35–0.66)	37	0.55 ± 0.01	0.54 (0.45–0.68)	40	–6.08	**<0.001**		
Anogenital distance, mm	15.16 ± 0.53	15 (7–22)	37	18.82 ± 0.26	19 (15–23)	40	–6.32	**<0.001**		
Midventral gland, mm^2	19.65 ± 1.71	16 (4–36)	37	30.55 ± 1.91	25 (9–64)	40			–3.66	**<0.001**

† Mean and error, ‡ median and limits of variability (in parentheses). N is the sample size. t_{St} are Student's t-test values for independent samples, $Z_{MW\text{-}U}$ is the Z-statistic in the Mann–Whitney test, and p is the probability of an erroneous prediction.

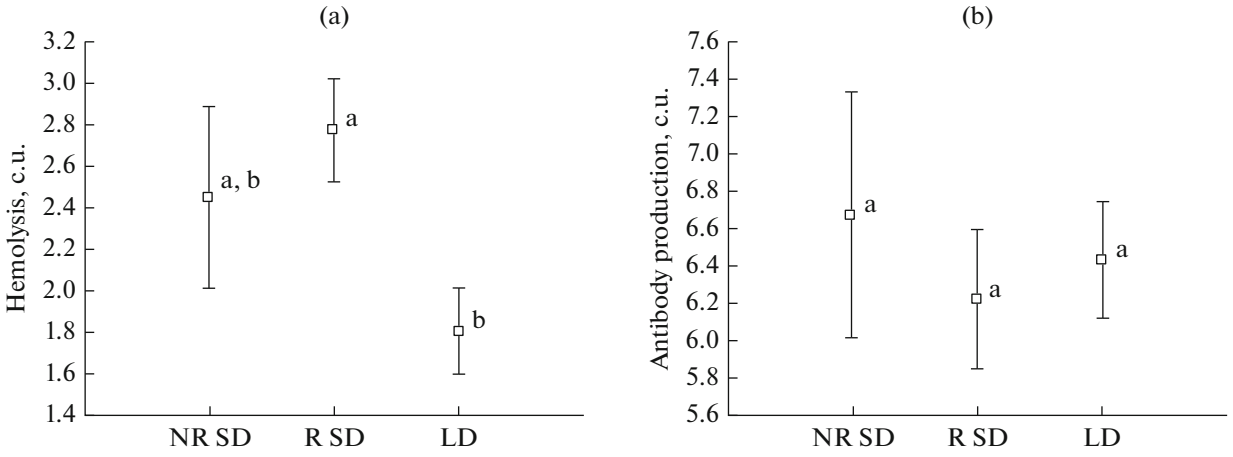

Fig. 3. Hemolytic activity of blood serum before immunization (a) and production of antibodies after immunization with sheep red blood cells (b) in SD and LD males: in SD nonresponders (NR), SD responders (R), and LD individuals at the end of the short and long day. Mean values and standard errors are given. Different letters on the graph indicate a statistically significant difference (Tukey HSD test: $p < 0.05$).

time, the hamsters of the SD group had significantly higher IHI indicators than the individuals of the LD group (Table 1). There were no differences between the groups in terms of AHI to immunization of hamsters with SRBC. The level of stress did not differ statistically in males of the SD and LD groups (Table 1). The experimental animals had a well-expressed individual variability of morphophysiological parameters associated with the reproductive system (Fig. 2). Comparison of IHI in SD nonresponders ($n = 9$), SD responders ($n = 28$), and LD individuals ($n = 40$) indicates the effect of photoperiod (one-way ANOVA: $F_{(2, 74)} = 4.607$, $p = 0.013$). At the same time, only SD responders (75.6%) significantly surpassed males of the LD group in terms of IHI indicators (Tukey HSD test: $p = 0.011$; Fig. 3a). There were no differences between all three groups in AHI (one-way ANOVA: $F_{(2, 74)} = 0.203$, $p = 0.816$; Fig. 3b).

Indicators of the immune status (humoral innate and adaptive) did not correlate with each other both within the SD and LD groups without distinguishing short-day nonresponders and short-day responders (SD: $R_{Sp} = -0.014$, $p = 0.93$, $N = 37$; LD: $R_{Sp} = 0.14$, $p = 0.39$, $N = 40$), and within the groups of SD nonresponders ($R_{Sp} = -0.30$, $p = 0.43$, $N = 9$) and SD responders ($R_{Sp} = 0.13$, $p = 0.52$, $N = 28$).

The indicator of the IHI status did not correlate with the generalized variable (PC1) characterizing the reproductive status for both SD and LD groups in general (SD: $R_{Sp} = 0.03$, $p = 0.84$, $N = 37$; LD: $R_{Sp} = -0.17$, $p = 0.29$, $N = 40$) and within the group of SD nonresponders ($R_{Sp} = 0.27$, $p = 0.48$, $N = 9$) and SD responders ($R_{Sp} = -0.26$, $p = 0.17$, $N = 28$). There were no correlations between the indicator of AHI and the generalized reproductive variable (PC1) in SD hamsters in general ($R_{Sp} = -0.12$, $p = 0.44$, $N = 37$) and within the groups of SD nonresponders ($R_{Sp} = 0.07$, $p = 0.86$, $N = 9$) and SD responders ($R_{Sp} = -0.09$, $p = 0.67$, $N = 28$). In the LD group, a significant, albeit weak, negative correlation was observed between the AHI and the generalized characteristic of reproductive status (PC1) ($R_{Sp} = -0.36$, $p = 0.024$, $N = 40$).

DISCUSSION

Keeping Campbell's dwarf hamster males under conditions simulating the winter photoperiod leads to a change in a number of morphophysiological parameters, indicating growth retardation and suppression of the reproductive system. These results are consistent with our earlier data on the dynamics of the morphometric parameters and reproduction of Campbell's dwarf hamsters kept throughout the year under the seasonally determined outdoor conditions (Khrushchova et al., 2023). The data obtained are also consistent with the classical ideas about the physiological adaptations to winter, in particular, in the Djungarian hamster (Scherbarth and Steinlechner, 2010).

The results we received in this study indicate an increase (compared to the LD group) in IHI together with a suppression of the reproductive function in SD males. When we excluded non-responding individuals from SD group, photoresponsive SD males significantly surpassed with a high support LD males in terms of IHI.

The strengthening of the innate component of the immune response in SD individuals may have various reasons, in particular, the conflict between immunity and reproductive system, the function of which is suppressed under short-day conditions. Recent reviews of publications on the effect of testosterone on the activity of different branches of immunity in different animal species generally confirm the negative effect of

testosterone on adaptive B-cell immunity, but data on the effect of testosterone on innate immunity are contradictory (Foo et al., 2016; Roved et al., 2017). The inconsistency of the results in relation to innate immunity may be related to the involvement of intermediaries in the regulatory mechanism. In particular, melatonin is one of such intermediaries, which concentration increases under short-day conditions (Goldman, 2001). In seasonally breeding mammalian species, in case of a short photoperiod, melatonin suppresses the seasonally dependent secretion of gonadotropins (Martin et al., 2008) and induces gonadal regression (Bartness et al., 1993). Increased melatonin secretion under short-day conditions may be a direct cause of testosterone decline (Hotchkiss and Nelson, 2002; Li and Zhou, 2015). In relation to immune functions, the effects of melatonin are diverse and their focus may be different (Prendergast et al., 2001; Martin, 2008; Calvo, 2013). However, the role of melatonin as one of the most important antioxidants and a significant immunomodulator is beyond doubt. It is possible that in our case the increased innate immunoresponsiveness in the SD regimen against the background of a reduced testosterone level could be mediated by melatonin.

On the other hand, while the winter immunity enhancement is based on an endogenous, genetically determined mechanism (Nelson and Demas, 1996; Nelson, 2004), keeping hamsters on a short day may well enhance not the costly adaptive humoral immunoresponsiveness, but the "inexpensive" innate protective mechanisms of rapid response in order to survive unfavorable winter conditions on the eve of future reproduction (McDode et al., 2016). In Campbell's hamster, when kept outdoors, reproduction starts already in the second half of winter (Khrushchova et al., 2023).

While the differences in the immunoresponsiveness of the SD and LD groups are based on the tradeoff between somatic investments and reproductive function (Martin et al., 2004, 2006, 2008), antibody production under conditions of long-day could be weakened by competition with the latter. Our assumption that, with the decrease in the activity of reproductive system, SD males will experience more intense immune response to SRBC, which requires costly resources both for the proliferation of B-lymphocytes and for antibodies production, has not been justified. However, although we have not obtained the expected differences in the intensity of response to SRBC between the SD and LD groups, the presence of a weak but statistically significant negative correlation between this parameter and the integral indicator of reproductive activity in the LD group does not contradict the hypothesis of a tradeoff between reproduction and immunity.

Indirect evidence in favor of the presence of competitive relations between the immune and reproductive systems in Campbell's dwarf hamster is given by the results of experiments carried out on animals originating from the eastern part of the range (Mongolia). SRBC immunization of castrated males did not lead to the expected increase in intensity of the immune response after repeated immunization. Admittedly, against the background of subsequent hormone replacement therapy, which raised the level of testosterone in castrated animals, the immunoresponsiveness to SRBC decreased partly compared with the control group treated with a placebo (Vasilieva et al., 2015). In another study, when comparing the intensity of antibody production in response to SRBC in males of the spring and autumn generations, the latter, having lower testosterone concentrations and testis size, nevertheless showed a tendency ($p = 0.13$) to a more intense immune response. In the same experiment, the delayed type of hypersensitivity response to cutaneous administration of PHA-mitogen was higher in males of the spring generation against the background of increased activity of the reproductive system (Rogovin et al., 2014b). As can be seen, the data of different experiments give ambiguous results, which indicates a complex system of relationships between immunity and reproduction. Another example of this is the results of experiments on selection in three generations of Campbell's dwarf hamsters for a low or high humoral immune response to SRBC, which, despite a heritable fixation of the trait, did not affect either AGD or the blood testosterone levels. Contrary to expectations, testosterone-dependent MVG in sexually mature males of two months of age was less developed in the group of low-immune animals (Rogovin et al., 2014).

It should be noted that in the present study the indicators of IHI and AHI in male Campbell's dwarf hamsters did not correlate with each other. This is yet more evidence of a different and independent response of the immune system to the day length. This is also supported by data on the immune response to winter conditions in other species, in particular, in the closely related Djungarian hamster. The experiments on this species in which the sickness behavior syndrome and the severity of peritonitis in response to various antigens were used to assess innate immunity led to the conclusions about its decrease under short-day conditions (Wen et al., 2007; Baillie and Prendergast, 2008; Pawlak et al., 2009). At the same time, SD males had a more pronounced inflammation response to intradermal administration of the DNFB (Bilbo et al., 2002; Prendergast et al., 2005). Although the intradermal injections of DNFB or plant mitogens (PHA, ConA) activate mainly innate immune response mechanisms (Vinkler et al., 2014), the intensity of the cutaneous DHT response may also indicate the state of adaptive T-cell immunity and is also used as a test for T-cell immunoresponsiveness (Bilbo et al., 2002; Tella et al., 2008). While the Djungarian hamster experienced an increase of cutaneous inflamma-

tion under short-day conditions (Bilbo et al., 2002), the adaptive B-cell immune response to the KLH antigen was suppressed during repeated immunization on a short day (Prendergast et al., 2004). The authors indicate also that the primary response to SRBC immunization, as well as to the KLH antigen, was the same in SD and LD hamsters, which is consistent with the results of our experiment. We cannot rule out that the differences between SD and LD groups in B-cell immunoresponsiveness after repeated immunization may also exist in Campbell's hamster. We abandoned repeated contact with SRBS in this work, because our task was to assess the constitutive innate immune response before the contact with the antigen. The species specificity may also affect the antigenic response. Thus, SD and LD male desert hamsters, in contrast to the Djungarian hamster, had no differences in the secondary immune response to SRBC (Vasilieva et al., 2020).

The data on the enhancement of the innate humoral immunity that we obtained are generally consistent with the the concept of winter immunity enhancement. We should also keeping in mind that in our experiment we placed animals in conditions that only partially imitated transition to the winter environment. It cannot be ruled out that under natural temperature, humidity, and food shortage animals may experience both a more significant internal redistribution of resources and deeper physiological changes affecting both the innate and acquired components of the immune system.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. In our study, we were guided by the recommendations given in "Guidelines for the Treatment of Animals in Behavioral Research and Teaching, ASAB/ABS 2012" (Buchanan et al., 2012) and Russian legislation. The study scheme was approved by the Commission on Bioethics at the Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, protocol no. 23 dated January 31, 2018.

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