**动物学报** 50(5):714 - 722, 2004
*Acta Zoologica Sinica*

# Endocrine status and scent attractiveness in male dwarf hamsters Phodopus sungorus injected with thymus-dependent and thymus-independent antigens *

Ekaterina KONDRATYUK, Ekaterina LITVINOVA, Eugene NOVIKOV, Dmitry PETROVSKI, Irene KOLOSOVA, Mikhail MOSHKIN**

Institute of Animal Systematics and Ecology, Siberian Branch of Russian Academy of Sciences, Frunze Street 11, Novosibirsk 630091, Russia

**Abstract** We studied the influences of immune activation by thymus-dependent (sheep red blood cells, SRBC) and independent (bacterial lipopolysaccharide, LPS) antigens on odor signals and endocrine status in dwarf hamsters. Administration of SRBS to mature males resulted in a drop of sexual scent attractiveness of soiled bedding collected during 5 days after injection. This effect was accompanied with a decline of fecal testosterone. Reduction of the male scent attractiveness after SRBC treatment had maximum manifestation in males of dwarf hamsters with low humoral immune response to this challenge. Contrary to the effects of SRBC, males injected with LPS showed an increase of scent attractiveness. Differences in the time that mature females spent sniffing olfactory stimuli (LPS *vs* control), correlated positively with differences in concentration of testosterone in feces collected from LPS and saline treated males. We discuss the adaptive meaning of the opposite olfactory effects, which induced by activation of the nonspecific innate immunity with LPS and by activation of specific acquired immunity with SRBC [*Acta Zoologica Sinica* 50 (5): 714 - 722, 2004].

**Key words** Dwarf hamster, *Phodopus sungorus*, Non-replicated antigens, Scent attractiveness, Testosterone, Cortisol

# 注射胸腺依赖和非胸腺依赖抗原雄性黑线毛足鼠的内分泌状态和气味吸引力 *

Ekaterina KONDRATYUK, Ekaterina LITVINOVA, Eugene NOVIKOV, Dmitry PETROVSKI, Irene KOLOSOVA, Mikhail MOSHKIN**

Institute of Animal Systematics and Ecology, Siberian Branch of Russian Academy of Sciences Frunze Street 11, Novosibirsk, 630091, Russia

**摘 要** 作者研究了胸腺依赖抗原(SRBC)(羊红血细胞, sheep red blood cells, SRBC)和非胸腺依赖抗原(细菌脂多糖, lipopolysaccharide, LPS)的免疫活化对黑线毛足鼠气味信号和内分泌状态的影响。成年雄鼠注射SRBS5天后, 其气味的性吸引力下降, 这种结果伴随着粪便中睾酮含量的下降。SRBC处理后, 雄性气味吸引力降低, 这在体液免疫反应低的雄性个体中最明显。与SRBC的作用相反, 注射LPS的雄性个体的气味吸引力增加, 成熟雌性个体用于嗅闻嗅觉刺激上的时间差异与经LPS和盐处理过的雄鼠的粪便中睾酮浓度的差异呈正相关。作者讨论了这两种相反的嗅觉效应的适应性意义[动物学报 50 (5): 714 - 722, 2004]。

**关键词** 黑线毛足鼠 非同源性抗原 气味吸引力 睾酮 皮质醇

Several studies showed the decline of the sexual scent attractiveness in male rodents infected with protozoan parasites *Eimeria vermiformis*, nematodes (*Heligmosomoides polygyrus*, *Trichinella spiralis*), tapeworm *Heminolepis diminuta* or influenza virus (Kavaliers and Colwell, 1993, 1995a, b;

Received Apr. 14, 2004; accepted July 15, 2004
* The research was funded by the grants from Russian Foundation for Basic Researches (RFBR) (No. 99-04-49927, No. 00-04-49210, No. 02-04-49253, and No. 03-04-48768) and Program of Russian Academy of Sciences "Origin and Evolution of Biosphere"
** Corresponding author. E-mail: mmp@eco.nsc.ru

Kavaliers et al. , 1997; Penn et al. , 1998; Klein et al. , 1999; Willis and Poulin , 2000). The ability to recognize and to avoid infected individuals is considered as a manifestation of the behavioral defense against parasites (Loehle , 1995; Penn and Potts , 1998; Kiesecker et al. , 1999). There are many ways for the parasite-dependent modification of host odor to occur (Penn and Potts , 1998; Moshkin et al. , 2000a). First , some parasites release their own volatile metabolites that can be detected by other animals (Welch , 1990). Second , the infections may affect the composition of commensal microbes in which influence individual odor (Sokolov et al. , 1992; Brown , 1995; Ushakova , 1995). Third , certain parasites can specifically reduce secretion of androgens (Fountain et al. , 1997; Morales-Montor et al. , 1999) , which plays important role in generation of the male sexual signals (Susumi , 1990; Ferkin et al. , 1994). Besides these parasite specific modifications of the host scent , which depend on taxonomy of the infection agents , their location and direct influences on the target cells of host etc. , we also proposed olfactory effects of parasites dealt with defensive immune response to an infection (Gerlinskaya and Moshkin , 1997). Parasite-derived antigens are known to activate the host immune system , including expression of the MHC genes (Kavaliers and Colwell , 1995a , b) which influence male odor (Yamazaki et al. , 1976; Penn and Potts , 1998). Due to the interaction of the immune and neuroendocrine systems , antigenic challenge modulates hormone secretion of such endocrine glands as gonads and adrenals (Moshkin et al. , 2000a) , which regulate chemical composition , production and release of scent marks (Drickamer and McIntosh , 1980; Susumi , 1990; Ferkin et al. , 1994). This way of the odor modification is an inevitable consequence for the many kinds of infections because practically each parasite releases foreign antigens into the host circulation.

Artificial antigenic challenge seems to be a powerful tool to address fundamental question on the own role of the immune defense in the alteration of sexual behavior and chemical signals found in infected animals (Moshkin et al. , 2000a; Staszewsk and Boulinier , 2004). Both thymus-dependent (T-dependent) antigens , which activate acquired immunity , and thymus-independent (T-independent) antigens , which activate mostly nonspecific innate immunity , are using in immuno-behavioral studies. For example , the evolutionary old T-independent antigen , bacterial lipopolisaccharides (LPS) , induces a repertoire of behavioral patterns collectively termed as sickness behavior (Dantzer , 2001). The key role in development of behavioral sick syndrome appears to be played by pro-inflammatory cytokines , which secretion is induced by LPS and other pathogen-associated molecular patterns (Dantzer et al. , 1996; Hacker , 2000). Administration of LPS or pro-inflammatory cytokines (IL-1 or TNFα) leads to decrease of sexual attractiveness of the treated laboratory rats (males or females) during their physical contacts with potential sexual partners (Avistur and Yirmiya , 1999). Using partner preference tests in study of the monogamous prairie voles *Microtus ochrogaster* and polygynous meadow voles *Microtus pennsylvanicus* , Klein and Nelson (1999) revealed that females of prairie voles , but not meadow voles , spent more time in the chamber with saline than LPS-treated males. At the same time , injection with LPS facilitates pair bond in females of prairie vole and does not affect on males of this monogamous species (Bilbo et al. , 1999). Behavioral effects of LPS were accompanied with increase in plasma corticosterone and decrease in testosterone (Klein and Nelson , 1999; Bilbo et al. , 1999). Although both hormones are belonged to physiological mechanisms regulated scent marking , the influence of the LPS administration on the scent attractiveness is still unclear.

Contrary to LPS , T-dependent antigens , such as sheep red blood cells (SRBC) , were used in several studies for elucidation of the role of the immune defense in generation of truthful signals about an infection (Saino et al. , 2000; Faivre et al. , 2003). Recently we have found a decline of scent attractiveness in SRBC-treated ICR male mice (Moshkin et al. , 2000b , 2001a , 2002). Then the similar effect of antigenic challenge was demonstrated on wild-derived house mice (Zala et al. , 2004). Males injected with a non-replicated strain of bacteria *Salmonella enterica* C5TS showed a significant decrease of scent marking and attractiveness of their scent marks to females. Since T-dependent antigens reduce androgens secretions (Barnard et al. , 1998; Moshkin et al. , 2002) and androgens strongly influence male attractiveness , we studied the olfactory effect of foreign antigens in gonadectomized and testosterone treated males (Litvinova et al. , in press). The stabilization of the circulating testosterone on the high level (not exceeding natural variation) abolished negative effect of SRBC on the male scent attractiveness. Among other reasons for the antigen-induced decline of male attractiveness , we cannot exclude the hypothalamic-pituitary-adrenocortical (HPA) axis , which is activated after injection with SRBC (Besedovsky and Sorkin 1975; Korneva and Shkinek 1988; Barnard et al. 1998; Moshkin et al. , 2001a , 2002). Hormones of this system secreted in response to stress stimuli can modify chemical signals in male and female mice (Drickamer and McIntosh , 1980; Novotny et al. , 1986; Cocke et al. 1993; Kavaliers et

al., 1998; Ma et al., 1998), and concentration of plasma corticosterone correlates negatively with odor attractiveness of male mice (Gerlinskaya et al., 1995; Moshkin et al., 2002).

In the present study on dwarf hamsters we compared the olfactory effects of the T-dependent and T-independent antigens, which activate different immune mechanisms. Usually immune response to many kind of parasite started from the macrophage reception of the evolutionary old T-independent antigens, which induce nonspecific innate immunity (Roitt et al., 1998). Then, if the infective agent successfully overcomes this nonspecific defensive line and successfully replicates, parasite derived T-dependent antigens induce acquired immune response, which is considered as the last line in the host defense against an infection (Roitt et al., 1998). Following to these considerations we can expect that activation of the acquired immunity rather than activation of the innate immunity should result in the decline of odor attractiveness.

We chose dwarf hamsters *Phodopus sungorus* for our experiments due to the wide use of this species in the study of chemical communication (Reasner and Johnson, 1987; Vasiljeva 1990; Wynne-Edwards et al., 1992; Novikov et al., 2004). A more specific reason was that odor mating choice plays an important role in breeding success of dwarf hamster couples (Evsikov et al., 2001). Females mated with preferred males had higher fecundity in comparison with females coupled with non-preferred males. SRBC and LPS were used as stimuli related to acquired and innate immunity, respectively. Because these antigens modulate hormonal secretion of the gonad and HPA, and hormones of these endocrine systems regulate the production, release and chemical composition of scent marks, we assessed concentrations of testosterone and corticosterone in feces collected before and after antigen administration. Radioimmunoasssay of fecal steroids was validated in special methodological studies on rodents and was recommended as an appropriate noninvasive approach to assess endocrine status (Gerlinskaya et al., 1993; Harper and Austad, 2000; Zavjalov et al., 2003). In the SRBC experiment, we collected soiled bedding for the olfactory tests during 5 days after antigenic administration. This time is enough to achieve maximum humoral immune response to novel antigens (Cunningham, 1965). Since peak of immune response to T-dependent antigens is accompanied with a decline of the gonad secretion and increase of adrenocortical activities (Besedovsky and Sorkin, 1975; Korneva and Shkinek 1988; Barnard et al., 1998; Moshkin et al., 2002), we sampled fresh feces at the 5-th night after SRBC administration to assess endocrine responses to this antigenic challenge. As for LPS, which induced fast immune and endocrine responses (Dantzer, 2001), we collected soiled bedding and fresh feces at the first and fifth nights after antigenic challenge.

## 1 Materials and methods

### 1.1 Animals and experimental protocol

Dwarf hamsters originated from West Siberia (Novosibirsk region) and have been bred in our laboratory for the past 10 years. We used 90 mature males and 47 females (5 - 7 month old). Two weeks before experiments, we caged all the individuals singly in plastic cages (20 ×50 ×10 cm). We kept males and females in separate rooms with artificial light-dark cycle 14L :10D, corresponding to the summer photoperiod (light was switched off at 17 : 00) and constant temperature 20 - 23 ℃. We provided dwarf hamsters with sawdust as bedding and *ad libitum* feeding (blend for hamsters: grain, seeds of sunflower and gourd, hips, eggs, carrot).

**1.1.1** Experiment 1 (T-dependent antigens) We injected 14 experimental males intraperitoneally with 0.5 ml of saline and 5 days later with 0.5 ml of SRBC ($2 \times 10^8$ cells). Both injections were done at 16: 00. We changed sawdust in male cages five days before and five days after antigenic challenge. We collected soiled bedding in glass vials (20 ml) and kept in freezer (- 20 ℃) up to olfactory choice test. Also we collected fresh fecal samples from the male cages during the night before SRBC-treatment and 5th night after the treatment. Feces were collected during 14 hours (from 17 :00 to 09 :00).

**1.1.2** Experiment 2 (T-independent antigens) We injected males from the experimental group ($n$ = 26) *i. p.* with LPS (L-4005, *E. coli*, serotype 055: B5, "Sigma") 50 μg/kg of body mass and males of control group ($n$ = 26) with saline at 15 : 00. We collected soiled bedding and fecal samples from each cage at the 1st and 5th nights after injection of LPS. In both times bedding and feces were sampled from 17 :00 (light off) to 09 :00.

**1.1.3** Experiment 3 (adrenocortical response to LPS) We injected 5 males with LPS (50 μg/kg) and 5 males with saline (control) at 15 :00. Three hours later we collected blood samples (100 μl) from the suborbital plexus. We sampled feces with 3 hour intervals from 18 :00 up to 18 :00 of the next day. We measured concentrations of cortisol in plasma and feces by radioimmunoassay.

### 1.2 Olfactory tests

In these tests we used soiled bedding collected before and after SRBC-treatment (experiment 1) or collected on first and fifth nights after injections with saline and LPS (experiment 2). We tested olfactory

preferences of female in plastic cage (45 cm × 15 cm ×15 cm) divided into three equal sections. Each lateral section was connected with the central section with the opening. Before the test we closed these openings by the doors and placed female in central section and two alternative tested samples - in lateral sections of the cage. In first experiment we used samples of soiled bedding collected before and after SRBC administration as alternative stimuli. In second experiment we used soiled bedding of control male and LPS-treated male. After removal of the fences we calculated total time spent by female in each section. Each test lasted 600 sec. We performed the tests from 18 :00 to 20 :00. We estimated preference of one of two odor stimuli by relative sniffing time (RST) that was calculated as:

RSTa = Ta/ (Ta + Tc) and RSTc = 1 - RSTa,

where: RSTa and RSTc are RST for the odor stimuli of antigen-treated and control males, consequently; Ta and Tc are the time, which female spent for inspection of the soiled bedding from cages of antigen-treated and control males.

Odor stimuli of each male were presented to 2 - 3 females in experiment 1 and to 1 female in experiment 2.

**1.3 Humoral immunity**

Five days after SRBC injections, males were sacrificed by cranium-cervical dislocation. We measured splenic antibody (plaque)-forming cells (AFC) by local hemolysis of the sheep erythrocytes (Cunningham, 1965) following the protocol described in Moshkin et al. (1998). We carefully removed the spleen and disrupted it in 5 ml of ice-cold medium 199 (pH = 7.3; Biopreparat Co., Novosibirsk, Russia). We prepared suspension of the cells using a 5-ml syringe to filter tissue through 1-mm mesh screen. We diluted the resulting suspension (1 :200) with 5 % acetic acid. We counted nucleated cells using a Gorjaev hemacytometer. The reaction mixture consisted of 500 μl spleen cell suspension, 500 μl washed SRBC ($4 \times 10^9$ erythrocytes/ml), and 500 μl of lyophilized guinea pig serum (Immunogen Co., PermR, Russia) resolved with 1 ml of isotonic sodium chloride solution. Cunningham chambers we prepared from glass microscope slides, loaded with 200 μl of reaction mixture, and incubated for 2 h at 37 ℃ before plaques were counted. We calculated the numbers of AFCs per individual.

**1.4 Radioimmunoassay of fecal testosterone and cortisol**

Fecal samples were dried at 30 - 40 ℃ and stored in a closed tube at room temperature. The concentrations of testosterone and cortisol in dry feces were measured by radioimmunoassay using Sigma antibodies (rabbit anti-testosterone; rabbit anti-cortisol) and Amersham labeled hormones ([1, 2, 6, 7-$H^3$] testosterone; [1, 2, 6, 7-$H^3$] cortisol). Steroids were extracted according to Gerlinskaya et al. (1993). Dry feces (60 mg) were homogenized in bidistilled water (3 ml) in a glass grinder. After centrifugation, supernatants were harvested and stored at - 20 ℃ until assayed. Supernatant (0.4 ml) was extracted with 3 ml ethyl ether; then 2 ml extract was removed, transferred to a new tube, vacuum dried at 55 ℃, and the residue was resuspended in 100 μl phosphate buffer, pH 7.4 (5 min, 60 ℃). Following Sigma's protocol, we added 100 μl diluted antiserum to the tubes with resuspended samples. After vortex and 60 min incubation at room temperature, 100 μl [$^3$H]-testosterone or [$^3$H]-cortisol diluted in phosphate buffer was added and samples were incubated again for 1 h at 37 ℃. After cooling in an ice-water bath, free and bound fractions of steroids were separated by 10 min incubations with 500 μl 1 % charcoal suspension and centrifuged at 2 500 g for 15 min at 4 ℃. The supernatants from each tube (500 μ) were added to scintillation cocktails and counted on a multi-purpose liquid scintillation counter (Beckman LS 6500).

The extraction yield was checked for every set of assays using $^3$H-labeled steroids and varied from 70 % to 76 % for both hormones. The assays were validated for use with hamster fecal steroid extracts by determining accuracy and parallelism. Sensitivity of the assay was determined from the 90 % confidence interval of zero standards that were 5 pg/tube for testosterone and 10 pg/tube for cortisol. The inter- and intra-assay variations were 9.9 % and 6.2 % for testosterone and 9.6 % and 5.5 % for cortisol, respectively. To determine parallelism, a five-point, two-fold dilution series of pooled fecal samples in phosphate buffer was prepared and compared with the standard curves of each steroid. There were no significant differences between the slopes of standard curves and the slopes of lines generated from fecal samples of assayed hamsters.

**1.5 Plasma cortisol**

We took blood samples from the suborbital sinus. The manipulations took no more than 2 - 3 min thus the stressful effect of handling was avoided. Following centrifugation (3 000/min, 15 min), the blood plasma was frozen (- 20 ℃) until assay. We measured cortisol concentration in 50 μl of plasma without ether extraction. We added 50 μl of phosphate buffer, pH 7.4, to each sample of plasma and the further procedures were as described above.

**1.6 Statistical analysis**

For comparison of scent attractiveness, levels of fecal testosterone and corticosterone determined before and after injection with SRBC, we performed

paired Student's *t*-test. In experiment 2 we used two-way ANOVA for evaluation of the effects of LPS and time after injection on RST and fecal steroids. Due to the small sample size in experiment 3, we used nonparametric Mann-Whitney's *U*-test for comparison of the cortisol concentrations in blood plasma and feces collected from control and LPS treated dwarf hamsters. Relationships between traits were estimated by means of Pearson correlation coefficients. We expressed data as mean ±*SE*.

## 2 Results

### 2.1 Influence of SRBC on the scent attractiveness and endocrine status (experiment 1)

In olfactory choice tests, where mature females inspected soiled bedding of the same individual, RST for soiled bedding collected before antigenic challenge was significantly higher than those collected after administration. Since females spent about equal time in the section with bedding of SRBC treated males (175.6 ±10.7 sec) and in the start section (155.4 ±10.7 sec, $P > 0.05$), we concluded that scent of these males was less attractive but not aversive. Reduction of scent attractiveness induced by SRBC administrations was accompanied with the decline of fecal testosterone after SRBC administration (Fig. 1). Concentration of cortisol did not differ before and after injection with SRBC (Fig. 1). Humoral immune response to SRBC varied in males of dwarf hamsters from 7 000 to 37 000 AFC per individual (18 958 ± 1 697). Since individual level of AFC correlated positively with RST of bedding collected after injection with SRBC (Fig. 2), we can conclude that antigenic challenge reduce scent attractiveness mostly in males with low humoral immune response.

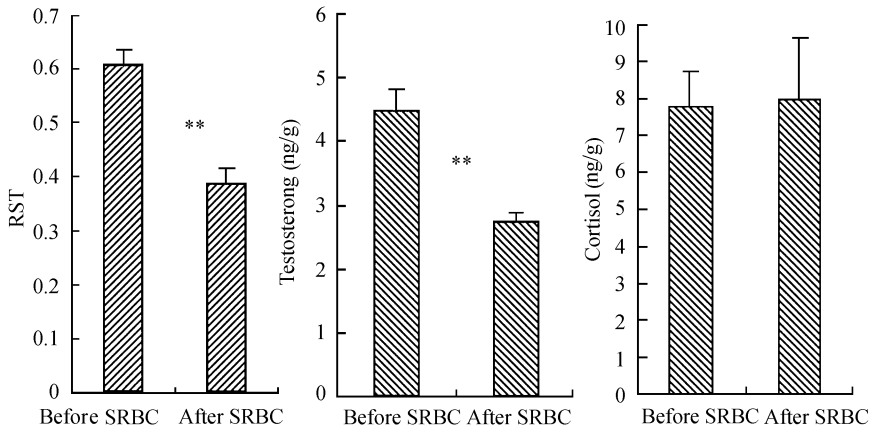


**Fig. 1 Relative sniffing time (RST) of soiled bedding, fecal testosterone and corticosterone in males of dwarf hamsters before and after injection with SRBC**

**: $P < 0.01$, paired Student's *t*-test; $t = 2.81$, $df = 39$ for RST and $t = 3.22$, $df = 13$ for testosterone.

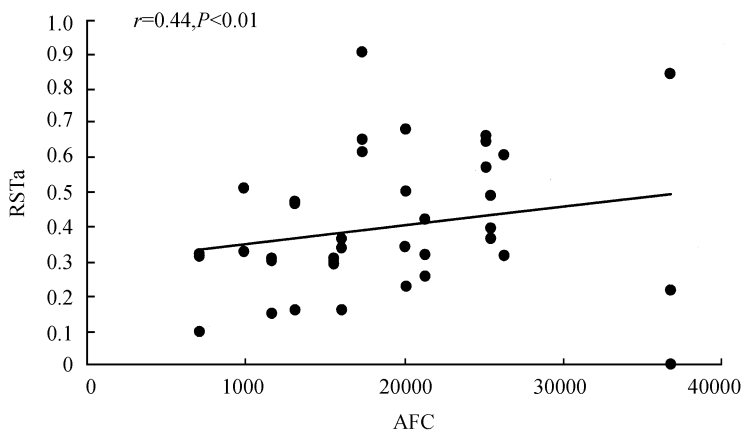


**Fig. 2 Correlation between relative sniffing time of antigen treated males (RSTa) and antibody forming cells (AFC) as an indicator of humoral immune response to SRBC**

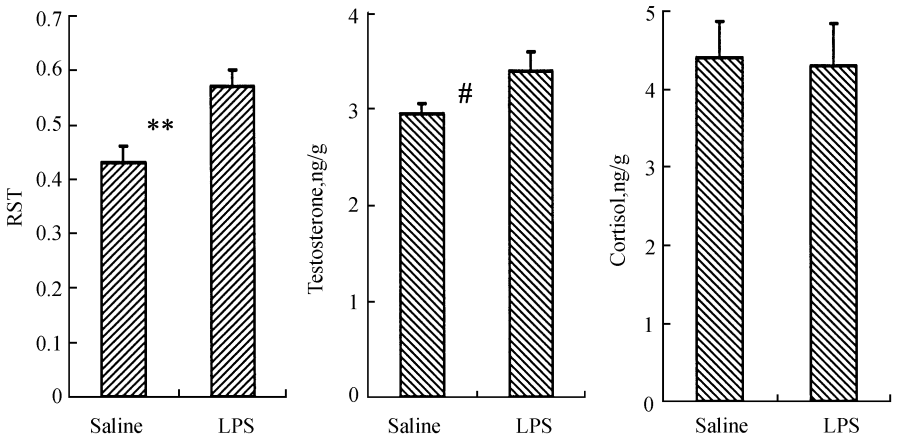


**Fig. 3　Relative sniffing time ( RST) of soiled bedding, fecal testosterone and corticosterone in males of dwarf hamsters injected with saline (control) and with T-independent antigen (LPS)**

**: $P < 0.01$, Student's $t$-test, $t = 2.76$, $df = 50$. #: $P < 0.1$, Student's $t$-test, $t = 1.87$, $df = 50$.

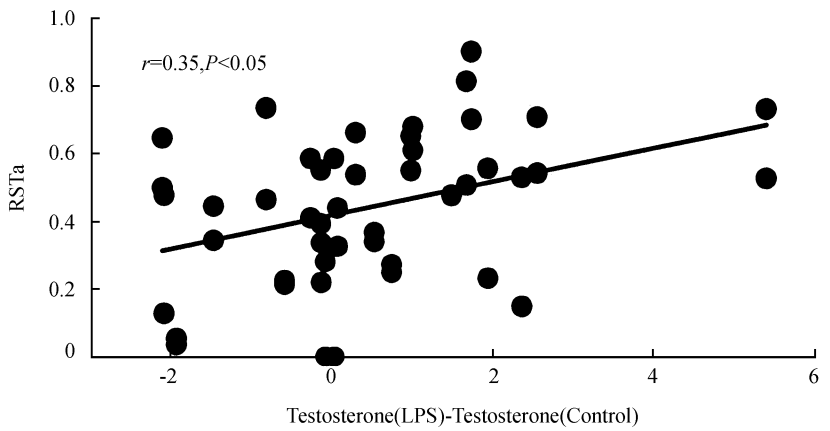


**Fig. 4　Correlation between relative sniffing time of antigen treated males ( RSTa) and difference in testosterone levels in pairs of tested males: concentration of testosterone in LPS treated males minus concentration of testosterone in control males**

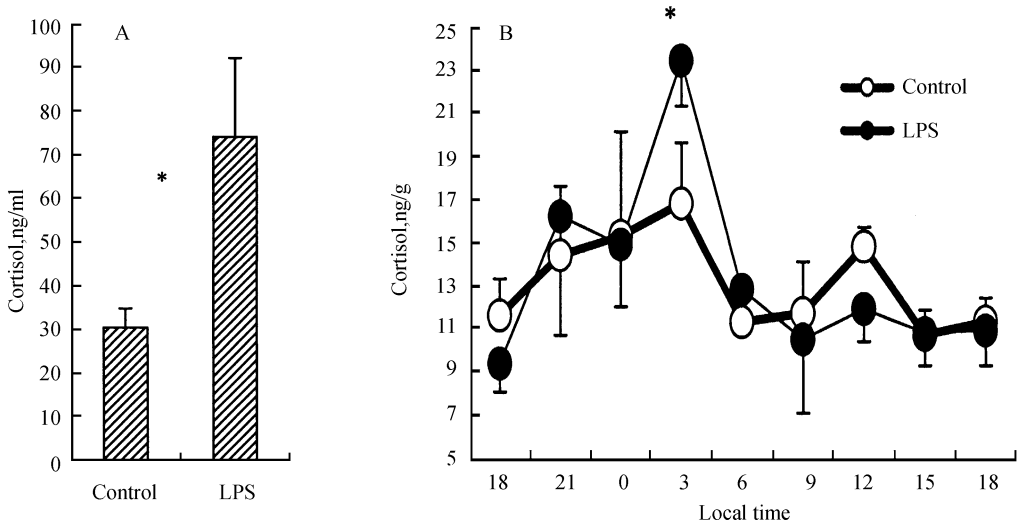


**Fig. 5　Influence of LPS on the HPA activity in males of dwarf hamster**

A. Concentration of cortisol in blood plasma collected 3 hour later on injection with saline (control) and with LPS.

B. Concentration of cortisol collected each 3 hour after injection with LPS.

*: $P < 0.05$ in comparison with control, Mann-Whitney $U$-test, $Z = 1.98$.

**2.2 Influence of LPS on the scent attractiveness and endocrine status (experiment 2)**

LPS-treatment significantly affected the odor attractiveness of males (two-way ANOVA, $F_{1,99} = 7.7$; $P = 0.0066$), whereas effects of the day of experiment and it's interaction with the LPS-treatment were non-significant. Regardless of the day RSTa significantly exceeded RSTc (Fig. 3, $P < 0.01$, paired Student's $t$-test).

As revealed by two-way ANOVA, neither LPS, day of sampling, nor interaction of LPS and day revealed any significant effect on the levels of fecal cortisol and testosterone. However concentration of testosterone in the LPS-treated group was slightly higher ($F_{1,99} = 3.5$, $P = 0.066$) than in the control (Fig. 3). On the other hand, RST of soiled bedding gained from LPS-treated male positively correlated with the difference in concentrations of testosterone between this male and control male ($r = 0.35$, $P < 0.05$), whose bedding was used as alternative stimulus (Fig. 4).

**2.3 Adrenocortical response to LPS (experiment 3)**

Concentration of cortisol in blood plasma sampled 3 hour after injection with saline or LPS was significantly higher in antigen-treated males of dwarf hamsters than in control males (Fig. 5A). We found significant differences in concentrations of fecal cortisol between experimental groups only 12 hours (at 03 a.m.) after injection with LPS or saline (Fig. 5B). Fecal cortisol progressively increased during 12 hours after administration of LPS or saline. Peak level exceeded the initial one (3 hour after injections) on the 13.0 ±0.5 ng/g in LPS treated group and on 5.2 ± 2.1 ng/g in control group (Mann-Whitney's $U$-test, $Z = 2.30$; $P = 0.022$).

## 3 Discussion

Our finding of a decline of scent attractiveness in SRBC treated males of the dwarf hamsters agrees with studies on laboratory mice (Moshkin et al., 2000b, 2001a, 2002) and on wild-derived house mice (Zala et al., 2004), which also demonstrated that activation of the immune system by non-replicated T-dependent antigens reduces male odor attraction. Olfactory effect of SRBC administration was accompanied by decrease of the fecal testosterone on the peak of humoral immune response to this antigenic challenge. In addition to urine, the dwarf hamster males release sexual pheromones through specific skin glands (Vasiljeva, 1990). Since androgens up-regulate functional activity of these glands (Vasiljeva, 1990), we can conclude that the decline of testosterone secretion is main cause of the reduction of scent attractiveness after SRBC administration.

Remarkably, the SRBC-induced drop of scent attractiveness was related to the magnitude of the humoral immune response to antigenic challenge. Positive correlation between RSTa and AFC showed that decline of the scent attractiveness in soiled bedding sampled after SRBC administration occurred mainly in individuals with low immunocompetence. So females of dwarf hamster seem to use chemical signals of antigen-challenged males to choose sires with the best acquired immunity.

Like in other rodents (Moshkin et al., 2001b), dwarf hamsters showed a rapid response of plasma glucocorticoids to injection with LPS, which was reflected by temporary increase of fecal cortisol. But lag time between stress stimulus (injection with LPS) and increase of fecal glucocorticoids in dwarf hamsters was considerably higher than in laboratory mice and bank voles (Moshkin et al., 2001b; Zavjalov et al., 2003). Probably, this species specificity is related with adaptation of dwarf hamster to dry food (grain, seeds etc.) that is available in their natural habitat. Due to the short-term rise of fecal cortisol we did not find statistically significant increase of the glucocorticoids in feces collected during 1st and 5th nights after injection with LPS. In contrast to studies on prairie voles and meadow voles (Klein et al., 1999), administration of T-independent antigens did not suppress androgen secretion. This contradiction could result from differences in studied species, serotypes of LPS, or dose, which was about 20 times less in our study in comparison with study of Klein and Nelson (1999). Moreover, fecal testosterone in dwarf hamsters tended to be higher in LPS treated males than in control males. And in parallel, we have found higher scent attractiveness in LPS treated males in comparison with control males. Differences of the sniffing time in paired samples of bedding correlated positively with differences in concentration of testosterone in feces of the donors of the soiled bedding.

Thus administration of T-dependent and T-independent antigens modifies reproductive chemosignals of males and subsequently affects female mating choice. Olfactory effects of both types of antigens coincided with changes of gonad secretion, which plays a key role in regulation of pheromone production by both uro-genital glands and skin glands (Vasiljeva, 1990). But the directions of the endocrine and olfactory effects were opposite for SRBC and for LPS. T-dependent and T-independent antigens stimulate different immune mechanisms. LPS as ancient antigens presumably stimulates evolutionary old nonspecific innate immunity, and SRBC induce evolutionary young acquired immune system. These data allows speculating that decline of the sexual attractiveness caused by

infection take part only in case when parasites break through the nonspecific immune barriers and activates the acquired immune system, which is considered as the last defensive line in host protection against parasites.

**Acknowledgements** We thank anonymous referees for helpful comments on the manuscript. We are also grateful to Robert Hudson for correction of the finally version of manuscript.

## References

Avistur R, Yirmiya R, 1999. Cytokines inhibit sexual behavior in female rats: synergistic effects of tumor necrosis factor alpha and interleukin-1. Brain Behav. Immun. 13: 14 - 32.

Barnard C, Behnke JM, Gage AR, Brown H, Smothurst, PR, 1998. The role of parasite-induced immuno depression, rank and social environment in the modulation of behaviour and hormone concentration in male laboratory mice. Proceeding of Royal Society Lond. B. 265: 693 - 701.

Besedovsky H, Sorkin E, 1975. Changes in blood hormone levels during the immune response. Proceeding of Society of Experimental Biology and Medicine 150: 466 - 470.

Bilbo SD, Klein Sl, Devries AC, Nelson RJ, 1999. Lipopolysaccharide facilitates patner preference in female prairie voles. Physiology and Behavior 68: 151 - 156.

Brown R, 1995. What is the role of the immune system in determining individually distinct body odours? Int. J. Immunopharm. 17: 655 - 661.

Cocke R, Moynihan JA, Cohen N, Grota LJ, Ader R, 1993. Exposure to conspecific alarm chemosignals alters immune responses in BALB/c mice. Brain, Behavior, and Immunity 7 (1): 36 - 46.

Cunningham AJ, 1965. A method of increased sensitivity for detecting single antibody-forming cells. Nature 207: 1 106 - 1 107.

Dantzer R, 2001. Cytokine-induced sickness behavior: where do we stand? Brain, Behavior, and Immunity 15 (7): 7 - 24.

Dantzer R, Bluthe RM, Auber A, Goodall G, Bret-Dibat JL, Kent S, Goujon E, Laye S, Parnet P, Kelley W, 1996. Cytokine actions on behavior. In: Rothwell NJ ed. Cytokines in Nervous System. Chapman and Hall, 117 - 144.

Drickamer LC, McIntosh TK, 1980. Effects of adrenalectomy on the presence of a maturation-delaying pheromone in the urine of female mice. Horm. Behav. 14 (2): 146 - 152.

Evsikov VI, Potapov MA, Novikov EA, Potapova OF, 2001. Adaptation of species on example interrelation parents-sons at mammals. Rus. J. Ecol. Evol. 1: 264 - 278 (In Russian).

Faivre B, Gregoire A, Preault M, Cezilly F, Sorci G, 2003. Immune activation rapidly mirrored in a secondary sexual trait. Science 300: 103.

Ferkin MH, Sorkin ES, Renfroe MW, Jonston RE, 1994. Attractiveness of male odors to females varies directly with plasma testosterone concentrations in meadow voles. Physiol. Behav. 55 (2): 347 - 353.

Fountain S, Holland MK, Hinds LA, Janssens PA, Kerr PJ, 1997. Intestittial orchitis with impaired steroidogenesis and spermatogenesis in the testes of rabbits infected with an attenuated strain of myxoma virus. J. Reprod. Fertil. 110: 161 - 169.

Gerlinskaya LA, Moshkin MP, 1997. Both virus infection and immunization by neutral antigens modify odor attraction and reproductive success in male mice. In: Taborsky B ed. Advance in Ethology. Contribution to the XXV IEC, Vienna, 6.

Gerlinskaya LA, Moshkin MP, Evsikov VI, 1993. The methodical approaches to estimation of stress level in the wild animals. Ecology 1: 97 - 100 (In Russian).

Gerlinskaya LA, Rogova OA, Yakushko OF, Evsikov VI, 1995. Female olfactory choice and its influence on pregnancy in mice. Advances in the Biosciences 93: 297 - 302.

Hacker H, 2000. Signal transduction pathway activated by CpG-DNA. Curr. Top. Microbiol. Immunol. 247: 77 - 92.

Harper JM, Austad SN, 2000. Fecal glucorticoids: a nonivasive method of measuring adrenal activity in wild and captive rodents. Physiol. Biochem. Zoology 73: 12 - 22.

Kavaliers M, Colwell DD, 1993. Aversive responses of female mice to the odors of parasitized males: neuromodulatory mechanisms and implications for mate choice. Ethology 95: 206 - 212.

Kavaliers M, Colwell DD, 1995a. Discrimination by female mice between the odours of parasitized and non-parasitized males. Proc. R. Soc. Lond. B. 261: 31 - 35.

Kavaliers M, Colwell DD, 1995b. Odours of parasitized males induce aversive response in female mice. Anim. Behav. 50: 1 161 - 1 169.

Kavaliers M, Colwell DD, Choleris E, 1998. Analgesic responses of male mice exposed to the odors of parasitized females: effects of male sexual experience and infection status. Behav. Neurosci. 112: 1 001 - 1 011.

Kavaliers M, Colwell DD, Ossenkopp K-P, Perrot-Sinal TS, 1997. Altered responses to female odors in parasitized male mice: neuromodulatory mechanisms and relations to female choice. Behav. Ecol. Sociobiol. 40: 373 - 384.

Kiesecker JM, Skelly DK, Beard KH, Preisser E, 1999. Behavioral reduction of infection risk. Proc. Nat. Acad. Sci. USA 96: 9 165 - 9 168.

Klein SL, Nelson RJ, 1999. Activation of the immune-endocrine system with lipopolysaccharide reduces affiliative behaviors in voles. Behav. Neurosci. 113 (5): 1 042 - 1 048.

Klein SL, Gamble HR, Nelson RJ, 1999. *Trichinella spiralis* infection in voles alters female odor preference but not partner preference. Behav. Ecol. Sociobiol. 45: 323 - 329.

Koreneva EA, Shkinek EK, 1988. Hormones and immune system. Leningrad: Nauka (In Russian).

Litvinova EA, Kudaeva OT, Mershieva LV, Moshkin MP, 2004. High level of circulating testosterone abolishes decline of scent attractiveness in antigen-treated male mice. Animal Behavior (In press).

Loehle C, 1995. Social barriers to pathogen transmission in wild animal populations. Ecology 76: 326 - 335.

Ma W, Miao Z, Novotny MV, 1998. Role of the adrenal gland and adrenal-mediated chemosignals in suppression of estrus in the house mouse: the Lee-Boot effect revisited. Biology of Reproduction 59: 1 317 - 1 320.

Morales-Montor J, Ridriguerz-Dorantes M, Cerbon MA, 1999. Modified expression of steroid 5 alpha-reductase as well as aromatase, but not cholesterol side-chain cleavage enzyme, in the reproductive system of male mice during *Taenia crassiceps* cysticercosis. Parasitol. Res. 85: 393 - 398.

Moshkin MP, Dobrotvorsky AK, Mak VV, Panov VV, Dobrotvorskaya EA, 1998. Variability of immune response to heterologous erythrocytes during population cycles of red *Clethrionomys rutilus* and bank voles *C. glareolus*. Oikos 82: 131 - 138.

Moshkin MP, Gerlinskaya LA, Evsikov VI, 2000a. The role of immune system in behavioral strategies of reproduction. J. Reprod. Dev. 46 (6): 341 - 356.

Moshkin MP, Litvinova K, Kolosova I, Mak VV, 2000b. The changes of odour attraction in male mice treated with non-replicated antigen. Reports of Russian Academy of Sciences 9: 277 - 279 (In Russian).

Moshkin MP, Kolosova IE, Novikov EA, Litvinova EA, Mershieva LV, Mak VV, Petrovskii DV, 2001a. Co-modulation of the immune function and the reproductive chemosignals. Asian-Aust. J. Anim. Sci. 14: 43 - 51.

Moshkin MP, Tamagawa A, Kolosova IE, Gerlinskaya LA, Iwakura Y, Endo Y, 2001b. Bevavioral and endocrine effects of endotoxin in wild type and IL-1 deficient male mice. Sick-sindrome or adaptive reaction? Reports of Russian Academy of Sciences 379 (4): 564 - 566 (In Russian).

Moshkin MP, Gerlinskaya LA, Morozova OV, Bakhvalova VN, Evsikov VI, 2002. Behavior, chemosignals and endocrine functions in male mice infected with tick-borne encephalitis virus. Psychoneuroendocrinology 27 (5): 603 - 608.

Novikov EA, Petrovsky DV, Kondratuck EY, Litvinova EA, Moshkin MP, 2004. Foraging and aggressive behavior of the dwarf hamsters *Phodopus sungorus* during physical or olfactory contacts with antigens treated conspecific. Zoological Journal 83 (2): 480 - 485.

Novotny M, Jemiolo B, Harvey S, Wiesler T, Marchlewska-Koj A, 1986. Adrenal-mediated endogenous metabolites inhibit puberty in female mice. Sciences 231: 722 - 725.

Penn DJ, Potts WK, 1998. Chemical signals and parasite-mediated sexual selection. Trens. Ecol. Evol. 13: 391 - 396.

Penn DJ, Schneider G, White K, Slev P, Potts WK, 1998. Influenza infection neutralizes the attractiveness of male odor to female mice *Mus musculus*. Ethology 104: 685 - 694.

Reasner DS, Johnson RE, 1987. Scent marking by male dwarf hamsters *Phodopus sungorus cambelli* in response to conspecific odors. Behavioral and Neural Biology 48: 43 - 48.

Roitt I, Brostoff J, Male D, 1998. Immunology. 5th edn. London: Mosby-Wolfe.

Saino N, Ninni P, Calza S, Martinelli R, De Bernardi F, Moller AP, 2000. Better red than dead: carotenoid-based mouth coloration reveals infection in barn swallow nestlings. Proc. R. Soc. Lond. B 267: 57 - 61.

Sokolov VE, Ushakova NA, Shubkina AV, Perfilova TV, 1992. Interaction between coat fur and bacterial microflora in dependence on physiological status. Proc. Russian Acad. Sci. 323: 980 - 983 (In Russian).

Staszewski V, Boulinier TH, 2004. Vaccination: a way to address questions in behavioral and population ecology? Trends in Parasitol. 20 (1): 17 - 22.

Susumi H, 1990. Social condition influences sexual attractiveness of dominant male mice. Zool. Sci. 7: 889 - 894.

Ushakova NA, 1995. Concerning the role of bacteria in chemical communications of animals. Adv. Biosci. 93: 253 - 257 (In Russian).

Vasiljeva NU, 1990. The function of specific skin glands. Functional and evolutionary aspects of marking behavior in *Cricetinae*. Ph. D. Thesis, Inst. Evol. Morphol. Anim. Ecol of Russian Ac. Sc., Moscow (In Russian).

Welch JB, 1990. A detector dog for screwworms (Diptera: Calliphoridae). J. Econ. Entomol. 83: 1 932 - 1 934.

Willis C, Poulin R, 2000. Preference of female rats for the odours of non-parasitised males: the smell of good genes. Folia Parasitologia 47: 6 - 10.

Wynne-Edwards KE, Surov AV, Telitsina AY, 1992. Field studies of chemical signaling direct observation of dwarf hamster *Phodopus* in Soviet Asia. In: Dory RJ, Muller-Schwarze D ed. Chemical Signals in Vertebrates **VI** New York: Plenum Press, 485 - 492.

Yamazaki K, Boyse EA, Mike V, Thaler EA, Mathieson BJ, Abbott J, Boyse J, Zayas ZA, 1976. Control of mating preferences in mice by genes in the major histocompatibility complex. J. Experim. Med. 144: 1 324 - 1 335.

Zala SM, Potts WK, Penn DJ, 2004. Scent-marking displays provide honest signals of health and infection. Behavioral Ecology 15 (2): 338 - 344.

Zavjalov EL, Gerlinskaya LA, Evsikov VI, 2003. Estimation of stress level in the bank vole *Clethrionomys glareolus* (Rodentidae, Rodentia) by fecal corticosterone. Zool. J. 82 (4): 508 - 513 (In Russian).