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Social interactions differentially affect reproductive and immune responses of Siberian hamsters

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Abstract

Social interactions can have pronounced effects on reproductive physiology and behavior in a wide range of species. Much less is known about the effects of social interactions on immunity. The goal of the present study was to test the effects of social interactions on both reproductive and immune responses in Siberian hamsters (*Phodopus sungorus*). Male and female hamsters were housed alone, in same-sex pairs or in mixed-sex pairs for 4 weeks. Animals were then immunized with the antigen keyhole limpet hemocyanin (KLH) and blood samples were drawn 5 days postinoculation. Reproductive tissue masses, testosterone, 17β-estradiol and cortisol concentrations were measured and immunity was assessed by measuring serum anti-KLH IgM, and mitogen-stimulated splenocyte proliferation. Male hamsters housed with a female had increased testosterone and anti-KLH IgM and elevated splenocyte proliferation compared with males housed alone. Female hamsters housed in same-sex pairs had increased serum IgM compared with females housed with males. Cortisol was elevated in both sexes housed with male conspecifics compared with the other experimental groups. Serum estradiol concentrations did not differ among females in any group. Collectively, the results of the present study suggest that social interactions can alter reproductive responses, but that these changes appear unrelated to changes in immunity. In contrast, the presence of a male conspecific elicits social-stress-induced elevations in serum cortsiol in both males and females, which is generally immunosuppressive. These data support the notion of social-stress-induced suppression of immune humoral and cell-mediated immune responses.

Keywords: Stress; Testosterone; Estrogen; Cortisol; Sickness; Trade-off; Energetics

1. Introduction

A wide range of environmental factors, both physical and social in nature, can exert profound influences on physiology and behavior. Considerable evidence exists demonstrating that environmental factors can affect reproductive function [1]. In many seasonally breeding rodent species, low ambient temperatures and reduced food availability present during winter can activate the hypothalamo–pituitary–adrenal (HPA) axis, leading to stress-induced suppression of a wide range of immune responses [2]. In addition, changes in ambient day length or temperature can trigger changes in gonadal sex steroids and subsequent changes in reproductive function and behavior [1].

In addition to physical factors, social factors also play an important role in the regulation of reproduction in several seasonally breeding rodent species [3]. For example, although maintenance in short "winter-like" day lengths inhibits reproductive function in individually housed deer mice (*Peromyscus maniculatus*), this response can be attenuated when males are housed with female conspecifics [4]. Similarly, exposure to either photoresponsive or photorefractory females is sufficient to attenuate gonadal involution in male Siberian hamsters (*Phodopus sungorus*) [5]. Exposure to a female conspecific during the transfer from short to long days also triggers an increase in luteinizing hormone secretion within minutes of exposure to females [5]. In other species, social interactions, rather than the

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ambient photoperiod, influence reproductive responses. For example, male aztec mice (*Peromyscus aztecus*) display marked increases in serum testosterone concentrations and reproductive organ masses in the presence of a female conspecific, whereas exposure to short days does not affect reproductive responses in this species [6]. In addition to changes in reproductive function, social interactions can exert a marked effect on behavior in hamsters. For example, male Siberian hamsters housed with a female display heightened aggression and glucocorticoid levels in response to an intruder male compared with individually housed males [7]. In contrast, male hamsters housed with opposite-sex littermates shortly after birth display less aggression directed towards novel pups when tested as adults than same-sex-housed males [8].

Social factors, in addition to their effects on reproductive physiology and behavior, can also exert marked effects on immunity (reviewed in Ref. [3]). Compared with reproduction, however, considerably less is known about the influences of social interactions on specific immune responses. In laboratory rats (*Rattus norvegicus*), social interactions during the initial postnatal period is critical for the subsequent health of adult males, as animals reared in a colony environment display an advantage over those not reared in isolation on a variety of immune measures [9]. Social interactions can also affect immune function in meadow voles (*Microtus pennsylvanicus*) exposed to mixed-sex pairings, with female meadow voles displaying higher antigen-specific antibody responses compared with their male partners [10]. Furthermore, social defeat increases serum glucocorticoids and suppresses antigen-specific antibodies in laboratory rats and Syrian hamsters (*Mesocricetus auratus*) [11,12]. Collectively, the results of these and other studies suggest that social factors play an important role in mediating both reproductive and immune responses in several mammalian species.

The effects of social interactions on reproduction and immune function have traditionally been examined independently of one another. More recently, however, the concept of energetic trade-offs between reproduction and immune function has been proposed (e.g., Refs. [13,14]). Specifically, it has been suggested that available energy is a finite resource and, during times of reduced energy availability, energy is reallocated from less critical physiological functions (e.g., reproduction) to those most critical for immediate survival (e.g., immunity). Conversely, during times of peak reproductive activity (e.g., breeding) when reproductive responses are maximal, physiological responses, including immunity, must be limited. The goal of the present study was to test the idea of potential trade-offs between reproduction and immune function in response to social interactions in male and female Siberian hamsters (*P. sungorus*). Assuming that activation of the reproductive and immune systems are energetically expensive and that energetic trade-offs between these two systems exist and are mediated, at least in part, by gonadal steroid hormones, we predicted that interactions with an opposite-sex conspecific would increase circulation concentrations of gonadal steroids and lead to concomitant decreases in humoral and cell-mediated immune responses.

2. Materials and methods

2.1. Animals and housing conditions

Adult (>60 days of age) male (n=18) and female (n=18) Siberian hamsters (*P. sungorus*) were obtained from our breeding colony maintained at Indiana University and were group housed (two to four animals per cage) at weaning. Two weeks before the start of the experiments, hamsters were individually housed in polypropylene cages (40×20×20 cm) in colony rooms with a 24-h light/dark (LD) 14:10 cycle (lights on at 0600 h EST). Temperature was kept constant at 20±2 °C and relative humidity was maintained at 50±5%. Food (Purina Rat Chow) and tap water were available ad libitum throughout the experiment. All animals were treated in accordance with the Indiana University Institutional Animal Care and Use Committee (IACUC).

2.2. Experimental procedures

At the start of the experiment, male and female hamsters were randomly selected and assigned to one of three groups: (1) individual housing in which males (M) or females (F) were housed alone, (2) same-sex housing in which two animals of the same sex (M/M or F/F) were housed together in the same cage, or (3) opposite-sex housing [M(/F) or F(/M)] in which one male and one female were housed together. Within each sex, mean body masses were similar among experimental groups prior to the start of the experiment. Prior to housing, male hamsters received bilateral vasectomies as described previously [10] to prevent successful insemination of females. To control for the effects of surgery on reproductive or immune function, all males received surgeries, regardless of their experimental condition. Animals were kept in their housing conditions for 4 weeks, during which the bedding material was left unchanged.

After 4 weeks of housing, all hamsters received a single subcutaneous injection of 100 μg of the novel antigen keyhole limpet hemocyanin (KLH; Calbiochem, San Diego, CA), suspended in 0.1 ml sterile saline (Day 0) and were then returned to the colony room. KLH is an innocuous respiratory protein derived from the giant keyhole limpet (*Megathura crenulata*). KLH was used because it generates a robust, nonreplicating antigenic response in rodents, but does not make the animals sick (e.g., inflammation or fever) [15]. Blood was drawn from the retro-orbital sinus on Day 5 postimmunization. This sampling period was chosen in order to capture peak immunoglobulin M (IgM) production (the primary immunoglobulin present in blood during an initial humoral response) during the course of the immune

response to KLH. On the day of blood sampling, animals were brought into the surgery room individually to minimize stress, lightly anesthetized with anhydrous diethyl ether vapors (Sigma, St. Louis, MO), and blood samples (~500 μl) were drawn from the retro-orbital sinus between 1000 h and 1200 h EST. Time from initial entry into the laboratory to the termination of individual blood sampling was <5 min. Samples were allowed to clot for 1 h, the clots were removed, and the samples centrifuged (at 4 °C) for 30 min at 2500 rpm. Serum aliquots were aspirated and stored in polypropylene microcentrifuge tubes at −80 °C until assayed.

2.3. Assessment of reproductive condition

Animals were killed by cervical dislocation immediately following blood sampling and paired testes, epididymides, and epididymal white adipose tissue (EWAT) were removed in males; ovaries, uterine horns, and parametrial WAT (PWAT) were removed in females. In addition, spleens were removed under aseptic conditions from all animals and immediately suspended in culture medium (RPMI-1640/HEPES). Tissues were cleaned of connective tissue and weighed to the nearest 0.1 mg by laboratory assistants naive to the experimental hypotheses and treatment assignments. In addition to reproductive tissue masses, blood serum testosterone and cortisol concentrations were determined in separate enzyme immunoassays (EIAs) from commercially prepared kits (Correlate-EIA™, Assay Designs, Ann Arbor, MI for testosterone and cortisol and Cayman Chemical, Ann Arbor, MI for estradiol). All instructions and protocols provided by the kits were followed and all samples were run in a single EIA for each hormone measured. These assays were previously validated for use with Siberian hamsters (G.E. Demas, unpublished data). The antiserum used was highly specific for the hormones measured; cross-reactivity with other steroid hormones was <0.01%. Intra-assay variability was <10% for all samples. The sensitivities of the assays are 3.82, 28.5, and 56.72 pg/ml for testosterone, 17β-estradiol, and cortisol, respectively.

2.4. Assessment of humoral immunity

To assess humoral immunity, serum anti-KLH IgM concentrations were assayed using an enzyme-linked immunosorbent assay (ELISA) according to a previously published method [16]. Microtiter plates were coated with antigen by incubating overnight at 4 °C with 0.5 mg/ml KLH in sodium bicarbonate buffer (pH=9.6), washed with phosphate-buffered saline (PBS; pH=7.4) containing 0.05% Tween 20 (PBS-T; pH=7.4), then blocked with 5% nonfat dry milk in PBS-T overnight at 4 °C to reduce nonspecific binding, and washed again with PBS-T. Thawed serum samples were diluted 1:20 with PBS-T, and 150 μl of each serum dilution was added in duplicate to the wells of the antigen-coated plates. Positive control samples (pooled sera from hamsters previously determined to have high levels of anti-KLH antibody, similarly diluted with PBS-T) and negative control samples (pooled sera from KLH-naive hamsters, similarly diluted with PBS-T) were also added in duplicate to each plate; plates were sealed, incubated at 37 °C for 3 h, then washed with PBS-T. Secondary antibody (alkaline phosphatase-conjugated-antimouse IgM diluted 1:2000 with PBS-T; Cappel, Durham, NC) was added to the wells, and the plates were sealed and incubated for 1 h at 37 °C. Plates were washed again with PBS-T and 150 μl of the enzyme substrate *p*-nitrophenyl phosphate (Sigma: 1 mg/ml in diethanolamine substrate buffer) was added to each well. Plates were protected from light during the enzyme–substrate reaction, which was terminated after 20 min by adding 50 μl of 1.5 M NaOH to each well. The optical density (OD) of each well was determined using a plate reader (Bio-Rad: Benchmark; Richmond, CA) equipped with a 405-nm wavelength filter, and the mean OD for each set of duplicate wells was calculated. To minimize intra-assay variability, the mean OD for each sample was expressed as a percent of its plate positive control OD for statistical analyses.

2.5. Assessment of cell-mediated immunity

To assess cell-mediated immunity, splenocyte proliferation in response to the T-cell mitogen, Concanavalin A (Con A), was determined using a colorimetric assay based on the tetrazolium salt 3 (4,5 demethylthiazol 2 yl) 5 (3 carboxymethoxyphenyl) 2 (4-sulfophenyl)-2*H*-tetrazolium (MTS) [17]. Splenocytes were separated from tissue by compressing the whole spleen between sterile frosted glass slides; separated cells were suspended in 4 ml of culture medium [RPMI-1640/HEPES supplemented with 1% penicillin (5000 μ/ml)/streptomycin (5000 Fl/ml), 1% L-glutamine (2 mM/ml), 0.1% 2-mercaptoethanol (5×10^{-2} M/ml), and 10% heat-inactivated fetal bovine serum]. Splenocyte counts and viability were determined with a hemocytometer and trypan blue exclusion. Viable cells (which exceed 95%) were adjusted to 2×10^{6} cells/ml by dilution with culture medium, and 50-μl aliquots of each cell suspension (i.e., 100,000 cells) were added to the wells of sterile flat-bottom 96-well culture plates. Con A (Sigma) was diluted with culture medium to concentrations of 40, 20, 10, 5, 2.5, 1.25, and 0.6 μg/ml; 50 μl of each mitogen concentration was added to the wells of the plate containing the spleen cell suspensions to yield a final volume of 100 μl/well (each in duplicate). Plates were incubated at 37 °C with 5% CO_2 for 48 h prior to addition of 20 μl of MTS/PMS solution [Promega; 0.92 mg/ml of phenazine methosulphate (PMS) in sterile Dulbecco's phosphate-buffered saline] per well. Plates were then incubated at 37 °C with 5% CO_2 for an additional 4 h. The optical density (OD) of each well was determined with a microplate reader equipped with a 490-nm wavelength filter. Mean OD values for each set of duplicates were used in subsequent statistical analyses. Dose–response curves were constructed using

group means of the mean OD values at each mitogen concentration and unstimulated cultures. All subsequent analyses were performed using data from lymphocyte stimulated with 10 μg/ml of Con A because this concentration was determined to be optimal (i.e., resulting in the largest lymphoproliferative response).

2.6. Statistical analyses

Body and tissue masses, hormone concentrations, anti-KLH antibody levels, and splenocyte proliferation were analyzed using separate one-way between-subjects analyses of variance (ANOVAs; SPSS, Chicago, IL). Differences between pairwise means were determined using Tukey HSD post hoc tests when the overall ANOVA was significant. Differences between group means were considered statistically significant if $p<0.05$.

3. Results

3.1. Body mass

There was a significant sex difference in body mass with males weighing significantly more than females across experimental manipulations ($p<0.05$; Table 1). Within males, hamsters housed in same-sex pairs weighed significantly more than males housed with females ($p<0.05$). Males housed alone had intermittent body weight compared with males housed in either same- or opposite-sex pairs, but did not differ significantly from either of these groups ($p>0.05$; Table 1). In contrast, there was no difference in body mass among females in any of the social groups ($p>0.05$; Table 1).

3.2. Reproductive and hormonal measures

Among males, paired testes and EWAT masses were significantly reduced in hamsters housed with a female compared with either males housed with another male or males housed alone ($p<0.05$ in both cases; Table 1). Among females, paired uterine horn masses were significantly smaller in females housed together compared with either females housed with males or females housed alone ($p<0.05$ in both cases; Table 1). Neither paired ovarian masses nor PWAT masses differed among females in any experimental group ($p>0.05$). Serum testosterone concentrations were significantly higher in males housed with females compared with either males housed in same-sex pairs or alone ($p<0.05$; Fig. 1a). Serum 17β-estradiol concentrations did not differ significantly among females in any of the experimental groups ($p>0.05$; Fig. 1b). Serum cortisol was significantly elevated in both males and females housed with males compared with the other experimental groups ($p<0.05$; Fig. 1c). Cortisol concentrations did not differ among any of the remaining experimental groups ($p>0.05$).

3.3. Immunological measures

Serum anti-KLH IgM was significantly lower in males housed in same-sex pairs compared with males housed with females or females housed in same-sex pairs ($p<0.05$ in both cases; Fig. 2a). In addition, there was a trend towards reduced (~50%) anti-KLH IgM in males housed together compared with males housed alone, but this comparison was not statistically significant. Serum anti-KLH IgM was significantly higher in females housed together compared with females housed alone ($p<0.05$; Fig. 2a). Females also displayed significantly higher IgM concentrations compared with males, but only when they were housed in same-sex pairs ($p<0.05$). Females housed with males did not differ in IgM levels compared to females housed either in same-sex pairs or alone ($p<0.05$). Serum anti-KLH concentrations were significantly negatively correlated with serum cortisol concentrations ($r=-0.435$, $p<0.05$).

Splenocyte proliferation was significantly lower in females housed with other males compared with females house either together or alone ($p<0.05$ in both cases; Fig.

Table 1
Mean (±S.E.M.) body and tissue masses (g) of male and female Siberian hamsters housed alone, in same-sex pairs, or opposite-sex pairs

	Body mass	Splenic mass	Testes mass	Epididymal mass	Ovarian mass	Utrine horn mass	EWAT mass	PWAT mass
Males								
Alone	40.72±1.44	0.058±0.005	0.713±0.023	0.247±0.014	–	–	0.839±0.088	–
Same-sex pairs	44.25±1.82*	0.064±0.004	0.697±0.022	0.244±0.017	–	–	0.751±0.109	–
Opposite-sex pairs	35.28±1.36	0.083±0.016	0.509±0.045*	0.240±0.014	–	–	0.478±0.040*	–
Females								
Alone	34.50±2.06	0.070±0.011	–	–	0.013±0.002	0.060±0.013	–	0.164±0.037
Same-sex pairs	33.00±1.49	0.080±0.010	–	–	0.014±0.003	0.014±0.003*	–	0.157±0.031
Opposite-sex pairs	32.77±3.10	0.074±0.009	–	–	0.011±0.003	0.081±0.012	–	0.151±0.040

* Significant difference, $p<0.05$.

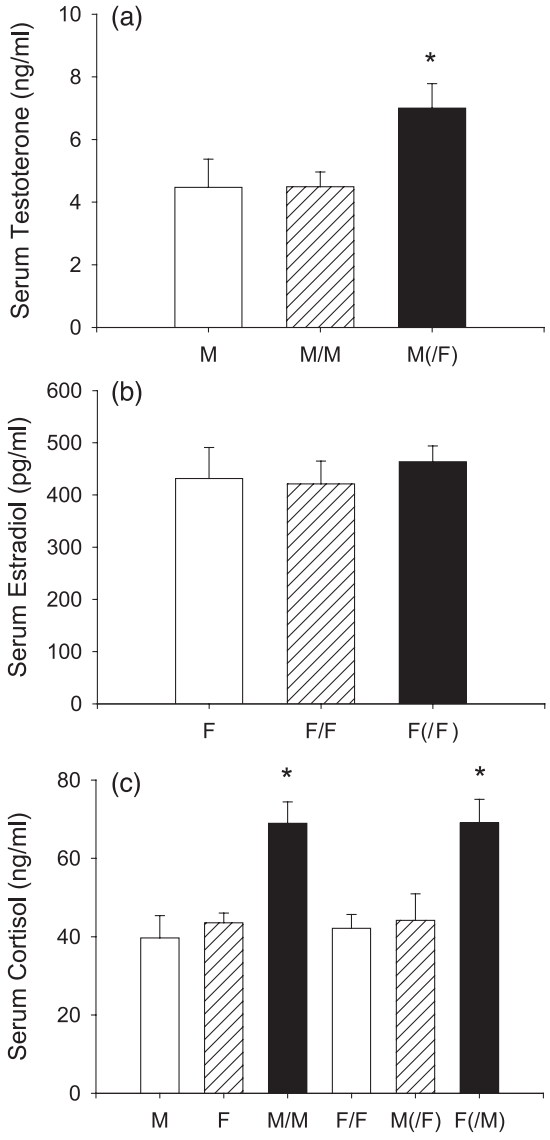

Fig. 1. Mean (±S.E.M.) serum testosterone (a), 17β-estradiol (b), or cortisol (c) concentrations of male or female hamsters housed alone (M or F), in same-sex pairs (MM or FF) or in opposite-sex pairs (M/M or F/F) for 4 weeks. Significant differences between pairwise means are indicated by an asterisk (*) if $p<0.05$.

2b). Splenocyte proliferation did not differ among males in different housing conditions ($p>0.05$). Males housed in same-sex pairs, however, did experience ~30% decrease in IgG compared with males alone or with females (Fig. 2b). Splenocyte proliferation was significantly negatively correlated with serum cortisol ($r=-0.564$, $p<0.05$). There were no significant differences in splenic masses across all experimental conditions ($p>0.05$ in all cases; Table 1).

4. Discussion

Consistent with several previous studies (e.g., Refs. [9,10]), social interactions influenced both reproductive and immune responses in male and female Siberian hamsters in the present study. In general, males housed in mixed-sex pairs displayed elevated serum testosterone concentrations, but elevated testosterone did not appear to affect either humoral or cell-mediated immune responses. Interestingly, however, males housed in same-sex pairs experienced elevated cortisol concentrations as well as decreased humoral and cell-mediated immunity. In contrast with males, housing conditions had no effect on serum estradiol concentrations in females, although uterine horn masses were reduced in same-sex-housed females. Females housed with a male conspecific had elevated serum cortisol concentrations and reduced cell-mediated immune responses. In contrast to males, however, humoral immunity was unaffected by elevated cortisol in females. Thus, although social interactions with an opposite-sex conspecific increased serum testosterone in males, but not estradiol in females, gonadal steroid levels did not appear to correlate with changes in immune function in either male or female hamsters. These results are inconsistent with the notion of an energetic trade-off between reproduction and

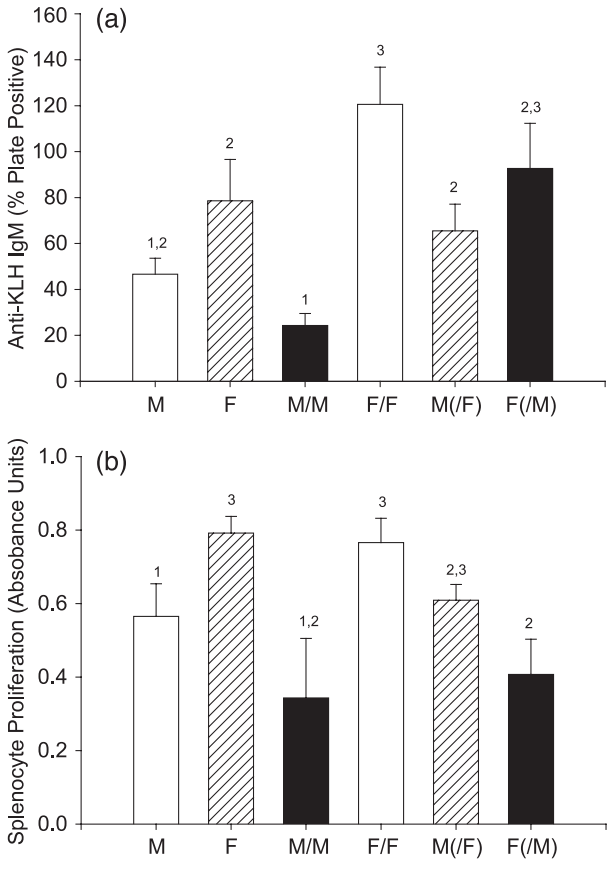

Fig. 2. Mean (±S.E.M.) serum anti-KLH IgM concentrations (expressed as a percentage of the plate positive control absorbance) (a) and splenocyte proliferation (absorbance units at 490 nm) in response to stimulation with the T-cell mitogen Concanavalin A (b) of male and female hamsters housed alone (M or F), in same-sex pairs (MM or FF) or in opposite-sex pairs (M/M or F/F) for 4 weeks. Columns sharing at least one same number are statistically equivalent. Columns with different numbers are statistically different ($p<0.05$).

immune function, at least within the context of social cohabitation. It is important to note, however, that food was available ad libitum for the duration of the study and thus, animals were capable of increasing food intake in order to compensate for increased energy demands. Thus, it is possible that trade-offs between immunity and reproduction may occur if food intake was restricted. Although this idea is intriguing, further research is required to test it.

Consistent with the immune data, serum cortisol concentrations were highest in males and females housed with other males and, in general, immune responses were also lowest in these experimental groups. Assuming that increased glucocortiocid secretion is indictative of a "stress response", these results suggest that, for males, same-sex housing is perceived as stressful and the resulting stress response (i.e., elevated cortisol concentrations) leads to decreased immune function. The increase in glucocorticoid secretion in males housed in same-sex pairs is likely due to heightened agonistic behavior relative to males housed with female or alone. In support of this hypothesis, a considerable amount of aggression was seen in males housed with same-sex conspecifics relative to males housed with females during daily behavioral observations taken throughout duration of the experiment (C. Johnson, G.E. Demas, unpublished observations). Agonistic interactions were particularly salient during the initial housing; however, sporadic aggressive encounters persisted through the duration of the experiment in male–male pairs. Although our observations suggest that agonistic interactions in male–male pairs may mediate stress-induced decreases in immunity, these behaviors were not rigorously quantified; thus, further studies are needed in order to provide more definitive support for this idea.

In female hamsters, same-sex housing did not significantly increase serum cortisol concentrations, suggesting that this form of social interaction was not perceived as stressful to females. In contrast, opposite-sex housing does appear to be stressful for females; females housed with males displayed elevated cortisol and decreased immune function compared to female housed alone or with another female. The precise causes of increased glucocorticoid secretion in these animals are not clear, as very little agonistic behavior was observed in either male or female hamsters in this experimental condition. It is well known, however, that social dominance hierarchies can lead to marked fluctuations in serum glucocorticoids (reviewed in Refs. [18–20]); the formation of such hierarchies in the present study may explain the increase in serum cortisol concentrations in females housed with opposite-sex pairs, although further studies are required to test this idea. Social interactions, whether with a male or another female, did not affect gonadal mass or circulating estradiol concentrations in females, although same-sex housing did reduce uterine horn mass. Compared with males, the effects of social interactions on female reproductive responses, at least those measured in the present study, appear to be more subtle.

In both male and female hamsters, the present results suggest that the immunological changes in response to social interactions are generally independent of changes in reproductive responses and circulating concentrations of gonadal steroid hormones. Specifically, changes in gonadal steroid hormones do not appear to be related to changes in either humoral or cell-mediated immune responses in either males or females. Rather, changes in circulating cortisol concentrations were more predictive of immunological changes in both sexes. Alternatively, the suppression of immune function seen in female hamsters housed with males may be mediated by the induction of pseudopregancy rather than stress-induced immunosuppression. Although we cannot rule out this possibility, it is not likely given that the significant increase in serum cortisol and reduction in immune response in females is consistent with response in male hamsters housed together. Thus, the stress-induced increase in circulating glucortiocoids in response to social interactions, whether due to the presence of same-sex (i.e., males) or opposite-sex conspecifics (i.e., females), likely mediates decreased humoral and cell-mediated immune responses in Siberian hamsters.

The results of the present study do not support the idea of a trade-off between reproduction and immune function in that decreased immunity does not appear to be mediated by concomitant changes in reproductive responses or circulating gonadal steroids in male and female hamsters. Alternatively, these results suggest that social-stress-induced increases in serum cortisol may be at least partly responsible for the effects of social interactions on immune function. Thus, the present findings are not inconsistent with an energetic trade-off model of immune function per se. The primary function of glucocorticoids is to mobilize energy in the form of glucose during times of metabolic stress. Thus, social-stress-induced decreases in immune function reported in the present study may be part of an adaptive physiological response in which moderate decreases in immune responsiveness are tolerated in order to conserve limited energy reserves and allow the reallocation of energy to more immediately critical responses, including behavioral interactions. In other words, the present findings are generally consistent with a trade-off between stress-induced physiological responses and immune function. Although this idea is intriguing, more research is needed to test it. Regardless of the precise mechanisms, the present findings support the idea that social interaction can exert strong influences on both reproductive and immune responses in male and female Siberian hamsters.

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