

Gretchen L. Hermes, Louis Rosenthal, Anthony Montag and Martha K. McClintock

Am J Physiol Regulatory Integrative Comp Physiol 290:273-282, 2006. First published Oct 6, 2005;
doi:10.1152/ajpregu.00368.2005

You might find this additional information useful...

This article cites 51 articles, 18 of which you can access free at:

<http://ajpregu.physiology.org/cgi/content/full/290/2/R273#BIBL>

This article has been cited by 2 other HighWire hosted articles:

Linking immune defenses and life history at the levels of the individual and the species

K. A. Lee

Integr. Comp. Biol., December 1, 2006; 46 (6): 1000-1015.

[Abstract] [Full Text] [PDF]

Sex and gender differences in pain and inflammation: a rapidly maturing field

K. J. Berkley, S. S. Zalcman and V. R. Simon

Am J Physiol Regulatory Integrative Comp Physiol, August 1, 2006; 291 (2): R241-R244.

[Full Text] [PDF]

Updated information and services including high-resolution figures, can be found at:

<http://ajpregu.physiology.org/cgi/content/full/290/2/R273>

Additional material and information about *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* can be found at:

<http://www.the-aps.org/publications/ajpregu>

This information is current as of January 6, 2009 .

CALL FOR PAPERS | *Sex and Gender Differences in Pain and Inflammation*

Social isolation and the inflammatory response: sex differences in the enduring effects of a prior stressor

Gretchen L. Hermes,^{1,2} Louis Rosenthal,⁴ Anthony Montag,⁵ and Martha K. McClintock^{1,2,3}

¹Institute for Mind and Biology and ²Departments of Comparative Human Development and

³Psychology, The University of Chicago, Chicago; ⁴Morris Institute, University of Wisconsin, Madison, Wisconsin; and ⁵Department of Pathology, The University of Chicago, Chicago, Illinois

Submitted 24 May 2005; accepted in final form 28 September 2005

Hermes, Gretchen L., Louis Rosenthal, Anthony Montag, and Martha K. McClintock. Social isolation and the inflammatory response: sex differences in the enduring effects of a prior stressor. *Am J Physiol Regul Integr Comp Physiol* 290: R273–R282, 2006. First published October 6, 2005; doi:10.1152/ajpregu.00368.2005.—Numerous epidemiological studies have demonstrated an association between persistent social isolation and “all-cause” morbidity and mortality. To date, no causal mechanism for these findings has been established. Whereas animal studies have often reported short-term effects of social isolation on biological systems, the long-term effects of this adverse psychological state have been understudied. This is the first animal study to examine the effects of long-term social isolation from weaning through young adulthood on an innate inflammatory response linked to numerous disease processes. Results presented here offer a plausible link between vulnerability to disease and social neglect. For socially isolated male and female Sprague-Dawley rats, a naturally gregarious species, formation of a granuloma in response to a subcutaneous injection of carrageenin (seaweed) was significantly delayed compared with the response of animals housed in single-sex groups of five. Significant sex differences, however, emerged when an acute prior stressor was superimposed on the experience of chronic social isolation. In this context, isolated females produced a more robust inflammatory response than isolated males. This sexual dimorphism at the nexus of chronic social isolation, acute stress, and inflammatory processes may account for the observation in humans that men with low levels of social integration are more vulnerable to disease and death than women.

innate immunity; cytokine; chemokine; corticosterone

PERSISTENT SOCIAL ISOLATION has been linked epidemiologically with high rates of “all cause” morbidity and mortality, carrying rises equal to that of cigarette smoking (4, 5, 27). We chose to examine the nonspecific inflammatory response, a fundamental and highly conserved immune process that is involved in multiple diseases and therefore could potentially link “all cause” morbidity and mortality to social isolation. Thus far, inflammation has been proposed by current biomedical research to be part of the etiology of a host of chronic diseases, including coronary and peripheral arterial disease (47), diabetes (29), lung disease (6), colorectal cancer (35), Alzheimer’s disease (57), and dementia (44). It is also the initial response to pathogens causing bacterial, viral, and parasitic infectious diseases. Here, we use an animal model of social isolation that

affords unique opportunities to test relationships between prolonged social isolation, stress, sex, and inflammatory processes.

As a measure of innate immunity, we tested the ability of Sprague-Dawley rats living in a group or isolate housing to produce a granuloma. Granuloma production is an innate nonspecific inflammatory response to a foreign substance, in this case, seaweed (carrageenin) in which a collection of metabolically active immune cells attempt to phagocytize and “wall off” a foreign substance independent of T-cell function (45). Macrophage-derived proinflammatory cytokines TNF- α and IL-1 β , as well as prostaglandins, produced as an initial response to the local exogenous irritant carrageenin, play important roles in increasing the permeability of local vessels causing leakage of proteinaceous fluid exudate into the area of the foreign substance. In addition, IL-1 β is an important chemoattractant factor for neutrophils, the most prominent immune cell in the acute phase of this response.

Exudate volume has been the research focus of granulomas induced by carrageenin and is thought to be an important, if not primary, biomarker of the inflammatory response (42, 43). Indeed, rat strains susceptible to autoimmune disorders have an impaired inflammatory response characterized by elevated exudate volume and exudate cellularity measured 10 days after granuloma induction (42, 43). However, exudate is only one aspect of the response, which begins with neutrophil and macrophage recruitment and ends with tissue healing. We sought to directly measure these cellular processes as critical indices of an organism’s capacity, pattern, and rate of healing, a process fundamental to resilience to many diseases.

Effects of social context on stress and immunity have been modeled by isolating laboratory rodents (1, 3, 33, 34). However, in most experimental models, social isolation has been brief, lasting from hours to a few weeks, and conceptualized as an acute stressor on which immunological manipulations were overlaid. Prolonged isolation has been understudied in rodent models, although we know in humans that long-term social isolation and loneliness predicts mortality and morbidity. For rats, isolation is also an atypical social condition and one we have hypothesized is perceived as largely negative by this gregarious species, which in naturalistic settings lives in large

Address for reprint requests and other correspondence: M. K. McClintock, Dept. of Psychology, The Univ. of Chicago, 5730 S. Woodlawn Ave., Chicago, IL 60637 (e-mail: mkm1@uchicago.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

colonies of closely spaced burrows with cooperative grooming, feeding, and rearing of offspring (12).

Effects of a long-term suboptimal social environment, such as isolation, are likely qualitatively different from chronic stress models in the rodent literature, in which repeated sessions of electric shock, cold swim, or restraint constitute the chronic condition. These stressful exposures are of limited duration and are not sustained, suboptimal contexts. Life-long social isolation of rats may relate more naturally, for example, to human studies of felt loneliness, bereavement, or the psychological impact of social disparities, where the individual must allocate biobehavioral resources for successful functioning, despite unremitting social challenge.

Researchers are beginning to elucidate these health risks—neural, endocrine, and immune implicit in sustained social challenges, such as isolation (36, 37). Persons providing long-term care for spouses with dementia, for example, report significantly greater loneliness and stress than controls. At the same time, they have higher levels of proinflammatory cytokines, more infectious illnesses, and exhibit slower wound healing (37). In some, but not all populations, loneliness is associated with elevated glucocorticoid levels (8, 54). To determine whether prolonged social isolation is best conceptualized as a stressor with its effects mediated by elevated basal or reactive glucocorticoids, we measured both basal and reactive corticosterone responses to a brief restraint stress.

Although we were interested in the effects of sustained social isolation on the proinflammatory processes underlying granuloma formation, we were also interested in its relationship to the acute stressors superimposed on this chronic social condition, as typically occurs in the natural lives of rats and humans. Chronic stress and depression can permanently alter immune function (15, 40), making organisms less resilient or more sensitized to additional stressors. In traditional models examining the impact of stress on immune function, stressors are repeated with cumulative stress effects measured proximate to the stressor (19, 20). But in the everyday lives of rats and humans, stressors are often single events, and the immune challenge may not occur simultaneously. For example, Johnson et al. (30) have shown that exposure to inescapable shock upregulated IL-1 β response to an injection of LPS 3 wk after the initial exposure, suggesting that the acute stressor leaves a “footprint” in immune function. Johnson’s work focused on T-cells as a central mediating mechanism for sensitization. Here we extend this concept to a nonspecific immunity that does not rely on memory in the immune system.

Because there are sex differences in response to acute stressors [female rats, for example have been shown to be more reactive than males (14)], we hypothesized that there would be sex differences in the interaction between social isolation and an acute stressor. In humans, a marked sexual dimorphism in the immune system is well established and is reflected in different immunological vulnerabilities of men and women (13). A recent study in humans demonstrated sex differences in the effect of psychosocial stressors on production of proinflammatory cytokines (48). Thus the fluid and cellular components of inflammation and related proinflammatory responses in the present animal model were hypothesized to have different sex-based patterns of development and elaboration.

MATERIALS AND METHODS

Animals and Social Conditions

Sprague-Dawley rats (60 females, 60 males) were weaned at 28 days and shipped to our laboratory from Charles River Laboratories. Upon arrival, rats of each sex were randomly assigned to one of two social conditions. Thirty females were housed as isolates in standard polypropylene hanging cages (26 \times 23 \times 22 cm) with wire mesh bottoms and slotted feeders; the other 30 females were housed in the same colony room in three groups of five, noncrowded in large steel cages (46 \times 61 \times 36 cm) with wire mesh cage bottoms and slotted feeders. Males were also housed as isolates or in groups in the same types of cages and kept in a separate colony room.

Colony rooms were maintained at 23°C, 50% relative humidity with a 14:10-h light-dark cycle (lights on at 1000 h central standard time). Food and water were available ad libitum. All rats remained in their respective social conditions for the duration of the experimental protocols from weaning (1 mo) through young adulthood (4 mo of age). Females were handled daily for 10–15 s to obtain measures of vaginal cytology, estrous cycle length, and reproductive state. Males were also handled daily to prevent a confounding between gender and the experience of human handling.

Ovarian Cycle Measure

Vaginal cytology was obtained daily for each female by vaginal lavage (middark phase). The changing proportion of cornified epithelial cells, nucleated epithelial cells, and leukocytes indicates estrous cycle phase and, in our hands, accurately predicts the day of the preovulatory luteinizing hormone surge in over 90% of cycles (22, 39). Estrogenization level was quantified as the percent of 14 days with only nucleated or cornified vaginal epithelial cells, a well-established bioassay for estrogen level. The Ovarian Cycle Regularity Index is a measure of the stability of cycle length within an individual (1 minus number of different ovarian cycle lengths/number of cycles observed) (39).

Protocol

At 100 days of age, half of the isolated and half of the group-housed animals of each sex received carrageenin, a nonspecific acute inflammatory immune challenge that induced a granuloma. The remaining animals were exposed to a single restraint stress and, 14 days later, were given the same immune challenge. This animal research was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Chicago.

Granuloma Induction Procedure

The inflammatory response was induced using the method of Selye with some modifications (51). At 100 days of age, the rats were injected subcutaneously between their shoulder blades with 12 ml of sterile air. This injection produced a well-defined cavity. An aliquot (4 ml) of 2% carrageenin (Sigma) in 0.9% NaCl solution was then injected into the air pouch. The induced nonspecific acute inflammatory response was assessed after 10 days, when it was optimal to determine individual differences in the rate of progress through the following stages of the inflammatory response.

Two to three days postcarrageenin challenge, the initial neutrophil response is replaced by macrophages and mononuclear inflammation. Throughout the acute and subsequent chronic stages of response, there is ongoing exudate formation and leukocyte recruitment to the local immune response with prostaglandins playing a less significant role than proinflammatory cytokines and chemokines, which continue to play a role in chemotaxis and exudation (46). In the advanced chronic phases of the response, 10 days postcarrageenin exposure, fibroblasts are present along with new blood vessels and collagen, which together form granulation tissue, a ring of collagenous tissue at the outermost

edge of the granuloma (23, 24). In the final stages of wound healing, levels of proinflammatory cytokines and chemokines typically return to basal levels.

Measured Components of the Granuloma Response

Exudate volume. Ten days postinjection animals were killed, the exudate was removed with a 1-cc needleless syringe while the granuloma was still in situ, and the volume was recorded.

Granuloma weight. The granuloma was then excised after separating it from the surrounding muscle and skin. It was weighed and fixed in formalin. Histological analysis revealed that in this chronic phase of response, 10 days postinduction, macrophages (predominantly) and fibroblasts (secondarily) contributed most significantly to the dry weight of the inflammatory tissue.

Healing Stage Scale

Tissue histology. We confirmed that granuloma formation, 10 days after carrageenin exposure, was at the phase when macrophage recruitment process was over, macrophages and exudate were being resorbed, and granulation tissue was forming. Random samples were taken from the fixed granuloma and embedded in paraffin. Serial sections were cut at 10 μ m and stained with hematoxylin and eosin.

Three independent ordinal scales were developed to assess histological stages of the granuloma response: number and organization of macrophages, thickness of the granuloma tissue (an index of macrophage recruitment), as well as the maturity of granulation tissue (richly vascularized connective tissue that is an index of wound healing). The sequential stages were quantified by: macrophage number (few = 1, sheets = 2, thick confluent sheets = 3); thickness of granuloma tissue (the magnification at which tissue sections occupied the bright field: $\times 20$ field = 0; $\times 10$ field = 1; $\times 4$ field = 2; $\times 2$ field = 3); and degree of granulation tissue development (active immature = 1; well-developed vascularized tissue = 2; and collagenous mature with fibrosis = 3). As expected, the final stage of granuloma tissue development on *day 10* was negatively correlated with macrophage number and thickness of granuloma tissue ($r = -0.67$, $P = 0.0001$, $n = 27$ specimens).

To quantify subsequent stages of healing visible on the surface of the granuloma, including vascularization and connective tissue formation (10, 11), we developed a five-point ordinal scale of the surface healing of the granulomas: 1 = few capillaries, uniform tan color, smooth surface; 2 = moderate vascularization, variable shades of tan, minimal bumpiness; 3 = well vascularized, pale pink, whorled, layering, and thickening; 4 = well-vascularized, rose color, thick, very bumpy, and layered; and 5 = highly vascularized, red, intense discoloration, whorled, thick, bumpy, and layered.

The surface and histological measures of healing were highly correlated ($r = 0.81$, $P = 0.004$). All scores of granuloma development/maturity were made by investigators blind to the animal's housing condition and history of restraint stress exposure.

Stressor Application and Corticosterone Measurement

To measure basal corticosterone and verify that the 30-min restraint stressor activated the hypothalamic-pituitary-adrenal axis and increased levels of plasma corticosterone in male and female rats, two blood samples were taken. Isolated animals that were part of the restraint stress testing were carried individually in their single cages from the colony room to an adjacent assay room at the onset of the dark phase, which is the beginning of the behavioral day. In group-housed animals, animals were lifted and carried one at a time to the assay room, and testing of all animals in the cage was done in sequence. In the assay room, each animal was placed in a restraint tube (Harvard Apparatus, Holliston, MA) (animal weight range: 150–400 g, 300–500 g, diameter: 75 mm, 100 mm; diameter inner tube: 62 mm, 85 mm) and when secured (<2 min) a 250- μ l volume

blood sample was obtained from the tail immediately and again after 30 min of restraint. Movement in the tube was highly restrictive, simulating the natural stressor of being trapped in a collapsed burrow.

Collected samples were allowed to clot and centrifuged (1,500 g) for 20 min. Serum was stored frozen at -70°C in microcentrifuge tubes until assayed for corticosterone with the use of a standard radioimmunoassay kit with slight modifications to increase sensitivity (ICN Biomedicals, Costa Mesa, CA). The three corticosterone assays performed in this study had an intra-assay variance of 9.4% and an interassay variance of 8.1%.

Cytokine Measures

Concentrations of macrophage chemoattractant protein (MCP-1), TNF- α , IL-1 β , and IL-6 in granuloma exudate were measured using commercially available kits. The limit of detection for MCP-1 ELISA (Biosource International, Camarillo, CA) was 8 pg/ml. For the TNF- α ELISA (BD Pharmingen, San Diego, CA), the limit of detection was 16 pg/ml. The limit of detection for rat IL-1 β ELISA (Biosource International) was 16 pg/ml. The limit of detection for rat IL-6 ELISA (Biosource International) was 8 pg/ml. All samples were diluted at least 1:2 for the ELISA. Instructions from the manufacturers were followed in all cases.

Statistical Analysis

Statistical analysis was conducted using StatView (SAS Institute, Cary, NC). Hormonal, histopathological, and cytokine data were summarized as means \pm SE analyzed by a two-tailed Student's *t*-test, ANOVA, or repeated-measures ANOVA.

RESULTS

Social Isolation and Stages of Inflammatory Response

Exudate. Social isolation significantly reduced the volume of exudate in the granuloma produced 10 days after the carrageenin challenge (isolates = 1.10 ± 0.21 ml vs. group-housed = 2.23 ± 0.32 ml, see Table 1 and Fig. 1A). Although there was no sex difference in this component of the innate inflammatory response (females, 1.71 ± 0.25 ml vs. males, 1.69 ± 0.33 ml; see Table 1), isolation had a greater inhibitory effect on males. Specifically, isolated males produced only one-third the exudate produced by males living in groups (isolated males 0.75 ± 0.34 ml vs. group-housed males, 2.51 ± 0.46 ml; $P \leq 0.01$). Isolation did not significantly reduce exudate volume in females (isolated females, 1.48 ± 0.22 ml vs. group-housed females, 1.94 ± 0.45 ml; $P \leq 0.14$).

Weight. Isolation did not alter the total tissue weight of the granulomas (isolated animals, 13.43 ± 1.73 g vs. group-housed animals, 13.01 ± 2.21 g). (See Fig. 2A and Table 1). Males in group-housing produced granulomas 48% larger by weight than those produced by group-housed females (group-housed

Table 1. Effects of social isolation and gender on three components of the granuloma response

| | Exudate | | | Weight | | | Healing | | |
|----------------------|---------|-------|--------------|--------|-------|--------------|---------|-------|--------------|
| | F | df | P | F | df | P | F | df | P |
| Housing | 8.44 | 1, 54 | 0.005 | 0.23 | 1, 40 | 0.635 | 5.90 | 1, 38 | 0.020 |
| Sex | 0.04 | 1, 54 | 0.843 | 5.59 | 1, 40 | 0.023 | 1.40 | 1, 38 | 0.244 |
| Housing \times sex | 2.89 | 1, 54 | 0.095 | 1.95 | 1, 40 | 0.171 | 0.28 | 1, 38 | 0.601 |

Boldface indicates statistically significant main effects, interactions, and trends.

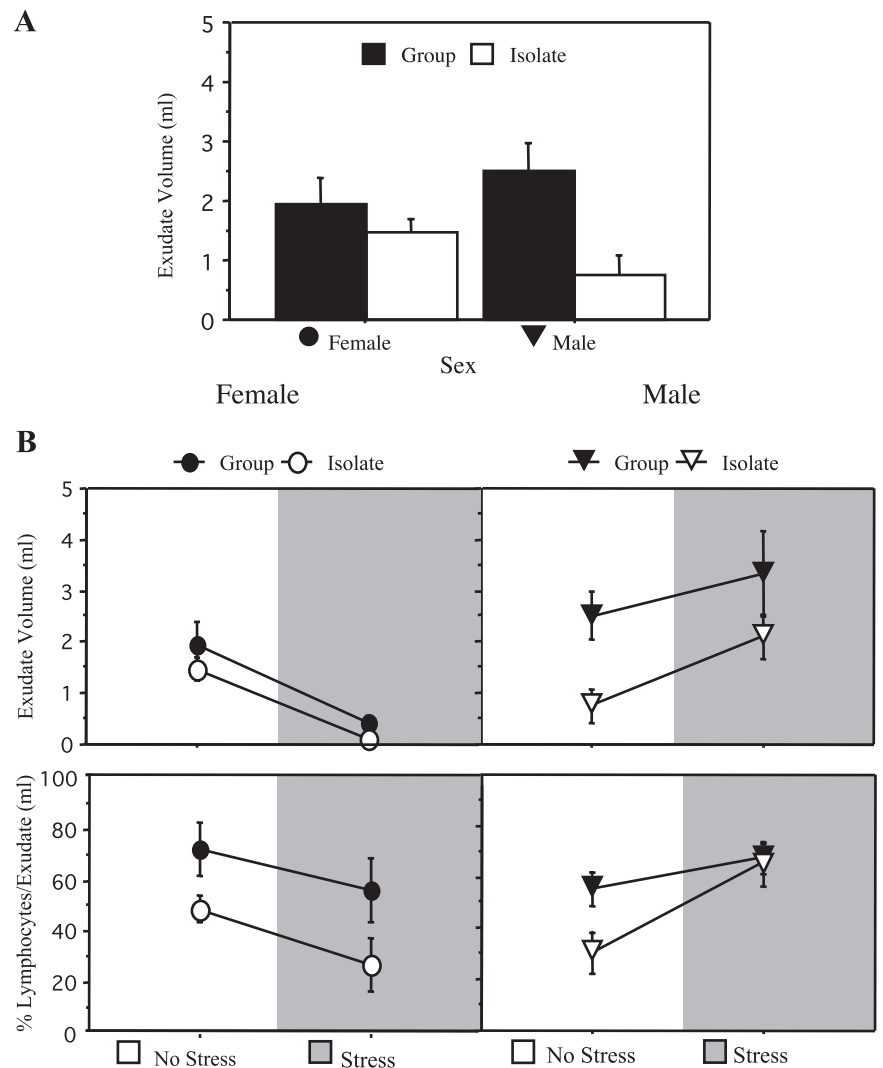


Fig. 1. *A*: main effect of prolonged social isolation on exudate volume without a prior stressor (** $P \leq 0.005$; see Table 1). *B*: sex differences in the effect of a brief prior stressor on exudate volume. See Table 2 for significant main effects and interactions of sex, stress, and isolation.

males, 20.4 ± 5.2 g; group-housed females, 9.9 ± 1.8 g; $P \leq 0.03$). However, in chronic isolation, no significant sex differences emerged (isolated males, 15.1 ± 4.0 g vs. isolated females, 12.4 ± 1.5 g; $P \leq 0.46$). Weight was correlated primarily with macrophage recruitment and secondarily with fibroblasts (see MATERIALS AND METHODS).

Healing. The granulation tissue (healing) of isolated animals showed less advanced stages of healing than in animals living in groups (isolated animals, 1.74 ± 0.25 vs. group-housed animals, 2.53 ± 0.23 , 5-point healing scale; see Fig. 3A and Table 1). There were no significant sex differences in the rate of healing (females, 2.00 ± 0.18 vs. males, 2.28 ± 0.41 , 5-point healing scale, and no significant interaction with isolation. See Table 1).

Effect of Social Isolation on Basal and Reactive Corticosterone

Basal levels. In both sexes, prolonged social isolation lowered basal corticosterone [main effect of isolation, $F(1,55) = 15.98$, $P \leq 0.0002$; lowered by $26.98 \mu\text{g/ml}$ corticosterone]. In absolute values, the effect of isolation was greater in females [female's reduction = $41.98 \mu\text{g/ml}$ corticosterone, male's reduction = $12.74 \mu\text{g/ml}$ corticosterone; interaction of sex and

housing $F(1,55) = 4.56$, $P \leq 0.04$]. However, as is well established, females have higher basal corticosterone levels than males [main effect of sex, $F(1,55) = 28.63$, $P \leq 0.0001$; sex difference = $36.41 \mu\text{g/ml}$ corticosterone]. Thus relative to basal levels of group-housed animals, there was no sex difference in the effect of isolation with a 59% reduction in basal corticosterone levels among females and a 77% reduction in males.

Reactive levels. Thirty minutes of restraint stress raised corticosterone levels in all animals, but particularly in females [main effect of sex $F(1,55) = 93.11$, $P \leq 0.0001$, see Fig. 4]. Social isolation significantly enhanced the corticosterone response, but only in females [isolated females 70% increase, Fisher's post hoc, $P \leq 0.002$; isolated males, 15% increase Fisher's post hoc, $P = 0.50$; main effect of housing $F(1,55) = 11.92$, $P \leq 0.001$; interaction between sex and housing, $F(1,55) = 9.31$, $P \leq 0.005$].

Social Isolation and Ovarian Function

Isolated female rats had shorter ovarian cycles than those of females living in groups (4.3 ± 0.2 days vs. 5.4 ± 0.5 days; $t = 2.0$, $P \leq 0.05$), which were equally regular (0.21 ± 0.05 vs. 0.31 ± 0.06 Regularity Index). Prolonged cycles were less

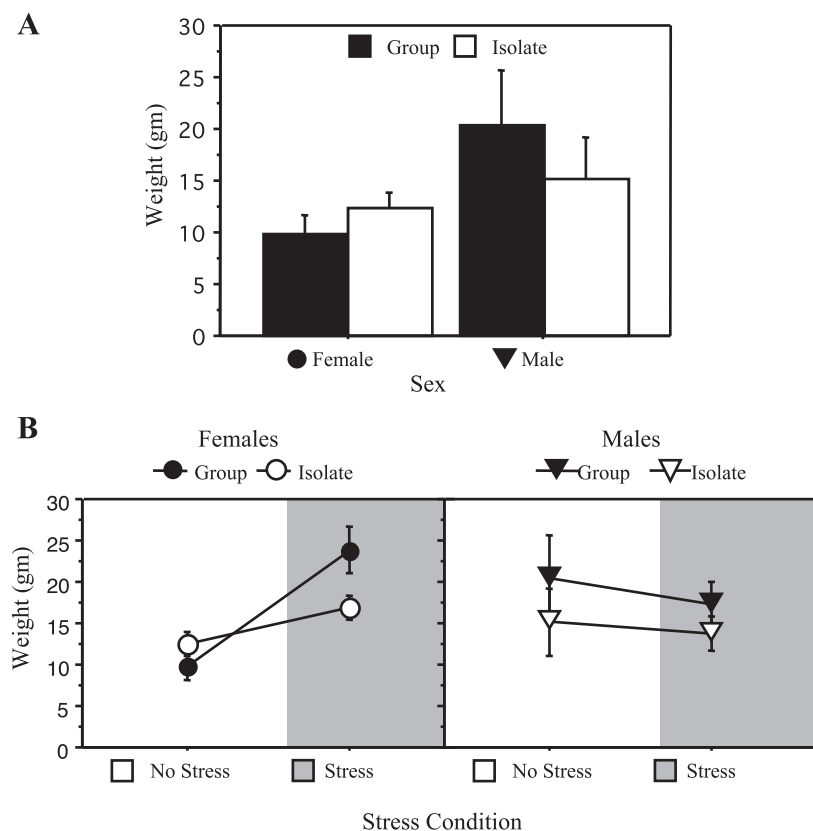


Fig. 2. A: effect of isolation on the magnitude of the inflammatory response and macrophage recruitment as inferred by weight (see MATERIALS AND METHODS. No stressor). B: sex differences in the effect of a brief prior stressor on weight. See Table 2 for significant interactions of sex, stress, and isolation.

common (5 or more days in length, 23 vs. 48% of cycles, $P \leq 0.05$), and they had more proestrus days during the 16 days before carrageenin exposure (3.8 ± 0.1 vs. 3.3 ± 0.2 , $t = 2.2$, χ^2 -test = 4.0, $P \leq 0.03$). Nonetheless, the level of estrogenization was the same in the two groups (48.7 ± 2.5 vs. 46.3 ± 2.4 of days), and isolated females were equally likely to be estrogenized on the day of granuloma induction (53.3 vs. 55.1% of days; χ^2 -test = 0.02, $P = 0.89$).

Sex Differences in Enduring Effects of a Prior Acute Stressor

Exudate. Two weeks after experiencing only one 30-min episode of restraint stress, female rats had a significant fivefold reduction in exudate formation present on *day 10* of the inflammatory response (no restraint stress, 1.7 ± 0.3 ml; prior restraint stress, 0.3 ± 0.1 ml; see Fig. 1B and Table 2). The effect was equally strong in isolated and group-living females [females only: main effect of prior restraint stress $F(1,54) = 31.81$, $P \leq 0.0001$; main effect of housing $F(1,54) = 2.28$, $P \leq 0.14$; interaction $F(1,54) = 0.09$, $P \leq 0.76$, see Fig. 1B and Table 2].

Males had the opposite response to a prior stressor, exudate volume was higher on *day 10* in both housing conditions [no restraint stress, 1.63 ± 0.33 ml; prior restraint stress, 2.80 ± 0.51 ml; main effect of restraint stress, $F(1,51) = 3.89$, $P \leq 0.05$; main effect of housing, $F(1,51) = 7.25$, $P \leq 0.01$; interaction $F(1,51) = 0.22$, $P \leq 0.64$; see Fig. 1B and Table 2].

Lymphocytes in exudates. Changes in number of lymphocytes recruited to local response paralleled changes in exudate volume caused by interaction of housing, sex, and stress (percentage of all cells in exudate). In socially isolated females, the concentration of lymphocytes was reduced by 26% [iso-

lated females, $37.87 \pm 6.15\%$ lymphocytes; group-housed females, $64.25 \pm 8.21\%$; main effect of housing, $F(1,54) = 6.95$; $P \leq 0.01$; see Fig. 1B]. With a history of a brief stressor among females there was a 19% reduction in numbers of lymphocytes in the exudate [females only: no stress, $60.20 \pm 6.12\%$ lymphocytes; prior restraint stress, $41.00 \pm 8.46\%$ lymphocytes; main effect of stress, $F(1,54) = 3.65$, $P \leq 0.06$; interaction, $F(1,54) = 0.08$, $P \leq 0.77$].

In males, a history of restraint stress had the opposite effect. On *day 10* postchallenge, there were 23% more lymphocytes in the exudate on *day 10* [males only: no stress, $42.83 \pm 5.65\%$ lymphocytes; prior restraint stress, $66.11 \pm 5.07\%$ lymphocytes; main effect of stress $F(1,52) = 9.87$, $P \leq 0.0003$, see Fig. 1B]. Lymphocyte recruitment to the granuloma site was not different for group or isolated males [males only, $F(1,54) = 3.49$, $P \leq 0.67$; interaction $F(1,52) = 2.27$, $P \leq 0.14$, see Fig. 1B].

Weight. Prior restraint stress also had a sexually dimorphic effect on macrophage recruitment (see Fig. 2B and Table 2). Females with a history of restraint stress produced heavier granulomas by *day 10* [females only: no restraint stress, 11.18 ± 1.15 g; prior restraint stress, 20.37 ± 1.69 g; significant main effect of restraint stress $F(1, 55) = 21.92$, $P \leq 0.0001$; main effect of housing $F(1, 55) = 1.21$, $P \leq 0.27$]. This effect was greatest in group-housed females that developed granulomas 140% larger than isolates [interaction of stress and housing $F(1,55) = 5.77$, $P \leq 0.02$]. A brief stressor also revealed that isolation reduced granuloma tissue formation by females, demonstrating that social isolation and a brief prior stressor had opposite effects on macrophage recruitment (isolated stressed female, 16.91 ± 1.49 ; group-housed stressed female 23.8 ± 2.82 , Fisher's post hoc test, $P \leq 0.0001$).

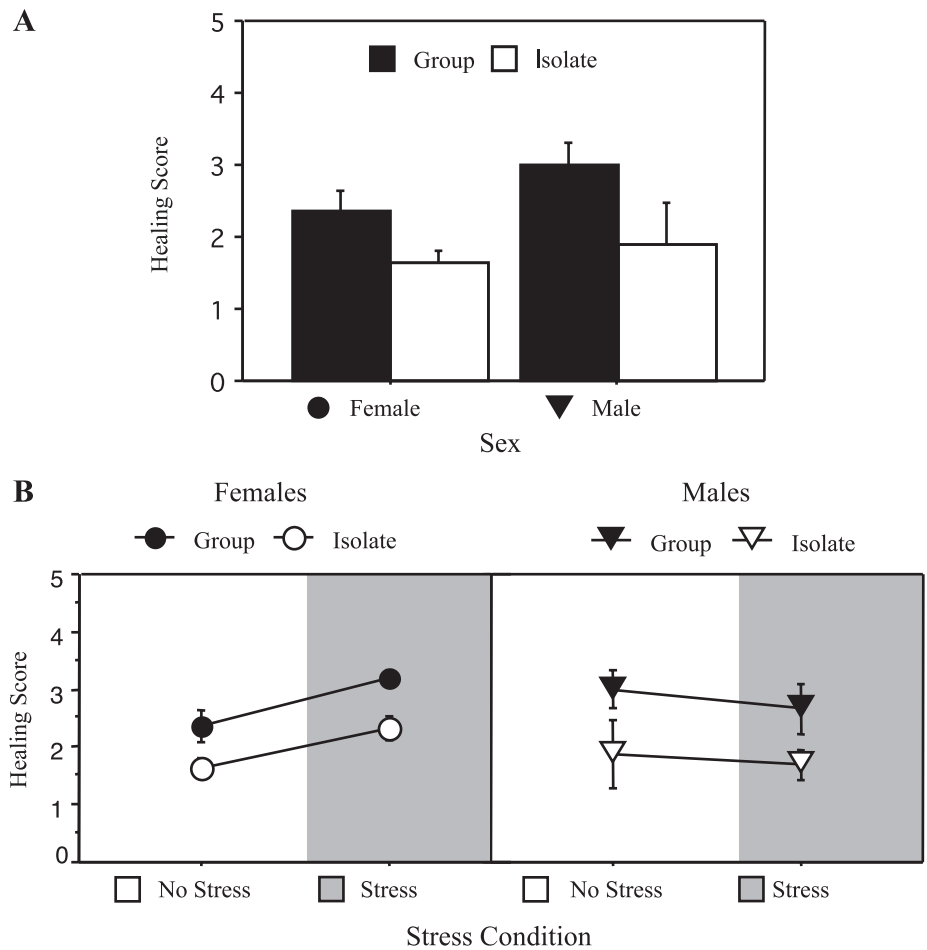


Fig. 3. A: main effect of prolonged social isolation on stage of healing without a prior stressor ($*P \leq 0.02$, see Table 1). B: sex differences in the effect of a brief prior stressor on healing. See Table 2 for significant interaction of sex and stress.

Unlike females, males did not respond to a history of restraint stress or isolation with significant changes in weight of the granuloma response [males only: no restraint stress, 16.15 ± 3.14 g; prior restraint stress, 14.33 ± 1.8 g; main effect of restraint stress, $F(1,27) = 0.42$, $P \leq 0.53$; housing $F(1,27) = 1.51$, $P \leq 0.23$; and interaction of housing and stress $F(1,27) = 0.06$, $P \leq 0.81$, see Fig. 2B and Table 2].

Healing stage. Females and males had opposite healing/granulation tissue responses to prior restraint stress (See Fig. 3B and Table 2). In females, a prior stressor facilitated wound healing in both group and isolated conditions [no restraint stress healing

stage = 2.00 ± 0.79 vs. prior restraint stress = 2.77 ± 0.15 ; females only, main effect of stress $F(1, 54) = 14.30$, $P \leq 0.0004$]. The stressor revealed significant detrimental effects of social isolation, although it enhanced healing in both housing conditions [restraint stress condition: isolate, 2.33 ± 0.21 vs. group-housed 3.2 ± 0.15 healing stage; Fischer's post hoc test, $P \leq 0.0006$; interaction between housing and prior restraint stress, $F(1, 54) = 0.13$, $P \leq 0.72$; see Fig. 3B and Table 2].

Unlike females, the experience of having been briefly stressed 3 wk earlier did not have enduring effects on males' rate of healing [no restraint stress, 2.29 ± 0.41 healing stage; prior restraint stress 2.16 ± 0.27 healing stage; males only: main effect of stress $F(1,29) = 0.32$, $P < 0.57$; see Fig. 3B and Table 2]. Chronic social isolation of males attenuated their wound healing with or without a stressor [isolated males, 1.79 ± 0.30 healing stage; group-housed males, 2.78 ± 0.30 ; main effect of housing, $F(1,29) = 5.10$, $P \leq 0.03$; interaction of stress and housing, $F(1,29) = 0.03$, $P \leq 0.88$].

Proinflammatory Cytokines and Chemokines: Kinetics of Response

Social isolation reduced IL-1 β production in exudate of both males and females (305.2 pg/ml in isolated animals; 858.2 pg/ml in grouped animals; see Fig. 5, A–C and Table 3). In contrast, there were marked sex differences in the enduring effects of brief restraint stress. A history of a brief stressor reduced IL-1 β to undetectable levels in

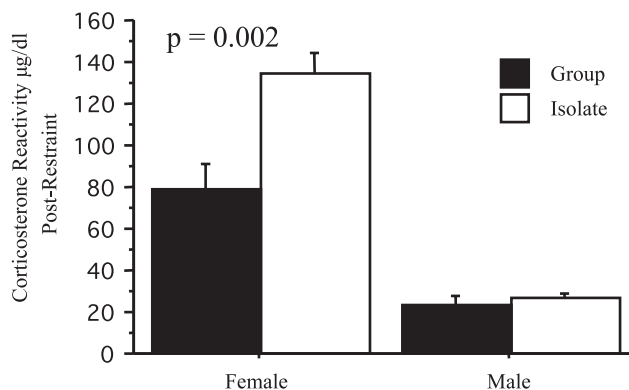


Fig. 4. Sex differences in corticosterone reactivity to 30 min of restraint stress.

Table 2. Components of granuloma formations; sex differences in enduring effects of a brief stressor in isolated and group housed rats

| | Exudate | | | Weight | | | Healing | | |
|------------------------|---------|--------|-------------------|--------|-------|--------------|--------------|-------|-------------------|
| | F | df | P | F | df | P | F | df | P |
| Housing | 9.86 | 1, 104 | 0.003 | 3.06 | 1, 82 | 0.084 | 17.15 | 1, 83 | <0.0001 |
| Sex | 15.72 | 1, 104 | <0.0001 | 0.21 | 1, 82 | 0.652 | 0.099 | 1, 83 | 0.754 |
| Stress | 0.31 | 1, 104 | 0.552 | 3.40 | 1, 82 | 0.069 | 1.31 | 1, 83 | 0.256 |
| Sex × stress | 17.79 | 1, 104 | <0.0001 | 9.44 | 1, 82 | 0.003 | 5.413 | 1, 83 | 0.022 |
| Housing × stress | 0.32 | 1, 104 | 0.573 | 1.07 | 1, 82 | 0.304 | 0.000 | 1, 83 | 0.993 |
| Sex × housing | 3.46 | 1, 104 | 0.066 | 0.35 | 1, 82 | 0.555 | 0.316 | 1, 83 | 0.575 |
| Sex × housing × stress | 0.09 | 1, 104 | 0.759 | 2.21 | 1, 82 | 0.141 | 0.113 | 1, 83 | 0.738 |

Boldface indicates statistically significant main effects, interactions, and trends.

isolated females and by > 50% in females living in groups (see Fig. 5, A–C and Table 3). In contrast, a history of brief stressor did not effect males significantly (although it increased IL-1 β by 10% among isolated males and 200% among group-housed males). These same sex differences in the effects of prior stress were detected in TNF- α concentration (see Fig. 5 and Table 3). Both IL-1 β and TNF- α levels were associated with %lymphocytes in the exudate on day 10 (confirmatory factor analysis Bartlett's $\chi^2 = 161.43$; $P < 0.001$) and independent of %neutrophils and MCP-1.

There were no detectable effects of isolation on MCP-1 levels in exudate, likely because the peak phase of macrophage recruitment had passed by day 10 (see Fig. 5 and Table 3). Nonetheless, social isolation revealed sex differences in the effects of stress: MCP-1 levels were lower in females, indicating that the macrophage recruitment phase was over, but still significantly higher in males, indicating that they

were still in the late stages of macrophage recruitment [isolates only: interaction between sex and prior stress $F(1, 35) = 4.59$, $P < 0.04$, prior stress $F(1, 35) = 1.29$, $P < 0.26$, sex $F(1,35) = 0.25$, $P < 0.62$].

There were no measurable levels of IL-6 in exudate, indicating that the granulomas were indeed a nonspecific inflammatory response, without evidence of antibody production or impurities in the carrageenin (53).

DISCUSSION

Effects of Social Isolation on the Nonspecific Inflammatory Response

In both females and males, social isolation from weaning through young adulthood delayed the innate inflammatory response. Although isolated and group-housed rats had re-

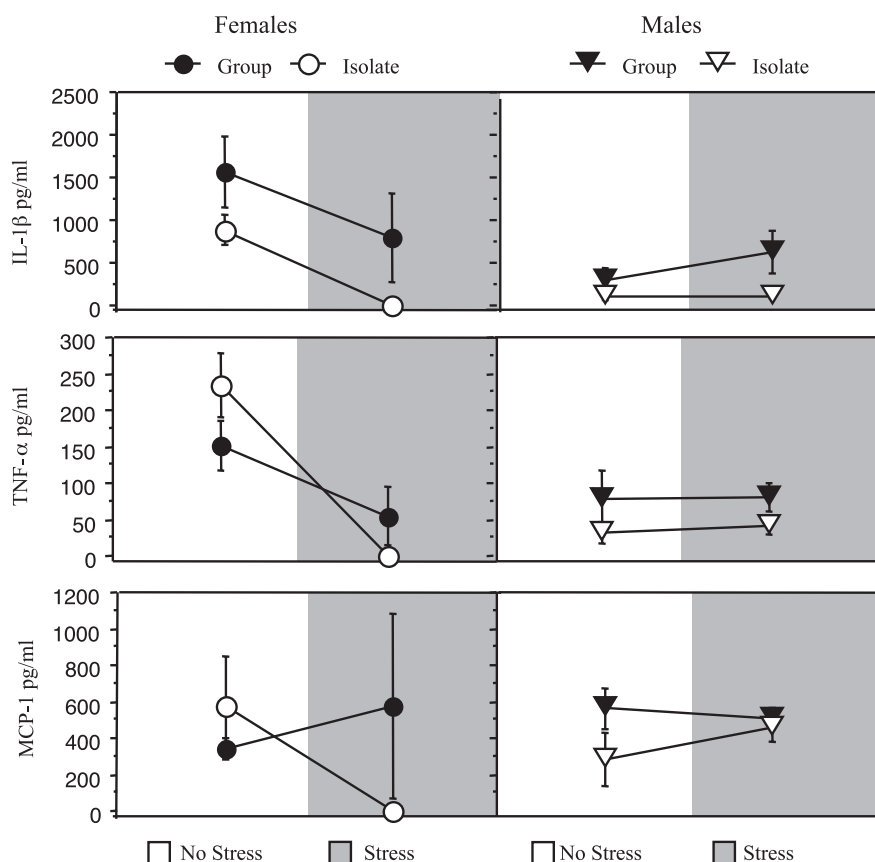


Fig. 5. Proinflammatory cytokine and chemokine concentrations and effects of prolonged social isolation and sex differences in response to a brief prior stressor. See Table 3 for significant main effects and interactions of sex, stress, and isolation.

Table 3. Cytokine and chemokine production; sex differences in enduring effects of a brief prior stressor in isolated and group housed rats

| | IL-1 β | | | TNF- α | | | MCP-1 | | |
|--------------------------------------|--------------|-------|--------------|---------------|-------|-------------------|--------------|-------|--------------|
| | F | df | P | F | df | P | F | df | P |
| Housing | 8.43 | 1, 83 | 0.005 | 0.41 | 1, 73 | 0.525 | 1.86 | 1, 79 | 0.177 |
| Sex | 7.80 | 1, 83 | 0.007 | 5.04 | 1, 73 | 0.028 | 0.44 | 1, 79 | 0.511 |
| Stress | 3.02 | 1, 83 | 0.086 | 12.29 | 1, 73 | 0.001 | 0.20 | 1, 79 | 0.655 |
| Sex \times stress | 7.00 | 1, 83 | 0.010 | 14.03 | 1, 73 | <0.0004 | 0.89 | 1, 79 | 0.347 |
| Housing \times stress | 0.33 | 1, 83 | 0.570 | 2.08 | 1, 73 | 0.154 | 1.37 | 1, 79 | 0.245 |
| Sex \times housing | 1.04 | 1, 83 | 0.311 | 1.58 | 1, 73 | 0.213 | 0.002 | 1, 79 | 0.965 |
| Sex \times housing \times stress | 0.09 | 1, 83 | 0.770 | 2.48 | 1, 73 | 0.120 | 4.46 | 1, 79 | 0.038 |

Boldface indicates statistically significant main effects, interactions, and trends.

cruited similar numbers of macrophages by *day 10* postcarrageenin exposure, their wounds were less resolved, a finding consistent with research showing delayed wound healing among individuals experiencing psychological distress (37, 41). Social isolation also significantly diminished the volume of exudate, a traditional indication of the magnitude of inflammation in response to carrageenin (42, 43).

In the chronic phase of a granuloma response to carrageenin, IL-1 β , not PGE, plays a primary role in maintaining permeability of the vascular endothelium as well as in promoting wound healing through its effects on angiogenesis, fibroblast proliferation, and chemotactic effects on immune cells (46). Thus both reduced levels of exudate volume and delayed wound healing in social isolation may be explained, in part, by the significantly reduced IL-1 β secretion present in the exudate, suggesting a link between social isolation and the production of proinflammatory cytokines. The similar levels of MCP-1 and granuloma weight suggest that social isolation by itself does not have the same effect on macrophage recruitment as it does on exudate production and wound healing.

In strains susceptible to inflammatory disease, such as Lewis rats, females produce more exudate in response to carrageenin, a response attenuated by tamoxifen, an estrogen receptor blocker (42, 43). In Sprague-Dawley rats, the sex difference in exudate production was evident only in the isolated rats, which had attenuated exudate formation. Isolated females actually had short ovarian cycles and more proestrus days on which estrogen rises sharply along with other ovarian steroids and gonadotropins. Thus an attenuated exudate production was associated with frequent exposure to rising estrogen, rather than less, as would be expected from the anti-inflammatory effects of tamoxifen. Clearly more work is needed to elucidate the role of naturally varying ovarian hormones in the regulation of exudate formation in the early phase of the inflammatory response.

Stress, Sex and the Kinetics of the Inflammatory Response

There were significant sex differences in the effects of a prior brief stressor on the production of granuloma tissue. With restraint stress 14 days prior to granuloma induction, females made more granuloma tissue, whereas males made less. A similar sexual dimorphism was reflected in measures of healing. Females that had been stressed healed more quickly than those that had not been stressed, whereas stressed males tended to heal more slowly than nonstressed males. Whereas the acute stressor accelerated wound healing in females, it reduced their

exudate levels with an expected reduction in levels of proinflammatory cytokines as the inflammatory process entered a remodeling phase and exudate volume was resorbed (23, 24, 42). Again males, in which wound healing was delayed by acute stress, exhibited greater exudate volumes and no decline in levels of proinflammatory cytokines or chemokines.

Although the functional significance of this sex difference in effects of stress on granuloma formation is beyond the scope of the present study, one hypothesis is that it evolved through selection on females in the context of maternal care and lactation. In maternal female rats, which must nurse their altricial pups for 3 to 4 wk, a protracted inflammatory sensitization by a stressor is likely to confer a survival advantage. While lactating, maternal rats become highly aggressive toward intruders and predators and are at high risk for wounding (55), particularly from neck bites that puncture the skin where the granulomas were induced. Stress-induced facilitation of the inflammatory response in threatened maternal rats would promote their healing and survival, with obvious benefit to her dependent offspring. Such sensitization could be mediated by corticosterone receptors (57), epinephrine (9, 16, 32), or cytokine (31) dynamics when pups are present.

Prolonged Social Isolation and Acute Stressors: the Same or Different?

When subjected to prolonged social isolation, both sexes exhibited reduced exudate volumes, fewer relative percentages of lymphocytes, and less tissue healing. Because isolated rats had lower baseline levels of corticosterone [a hormone normally considered to be more immunosuppressive (10)] than those living in groups, we cannot completely explain delayed granuloma formation and healing in the isolate condition. However, chronically low stress hormone levels may upregulate glucocorticoid receptors, particularly on immune cells (52), so that its functional effect is magnified during an acute stressor. The question of the role of stress hormone is even more complex when we consider that female rats, when stressed, had a more highly reactive corticosterone response to the brief stressor than did males, yet had more accelerated healing. Mechanisms underlying this response among females may contribute to the immunoenhancing effects of concomitant acute stressors on a delayed-type hypersensitivity response (18, 19, 20) as well as stress sensitization of the inflammatory response to LPS (31), both of which involve nonspecific inflammation as well as T-cell participation.

Results from this study indicate that the immunological outcome of a prolonged adverse social condition are not comparable to the enduring effects of an acute physical stressor. For males and females, as we have discussed, chronic social isolation retarded the inflammatory process. When an acute brief stressor was applied however, males and females had opposite responses. In females, the acute stressor enhanced the inflammatory response (with overall reductions in exudate volume, recruitment of lymphocytes to the local response and more advanced healing scores), whereas in males, the application of the acute stressor suppressed the inflammatory response (decreasing exudate resorption, cellular recruitment, and delayed healing).

Tend and Befriend: Sex Differences in the Robustness of Inflammatory Response

The enhanced stress-driven inflammatory response among females may speak to other questions about sex differences in neuroendocrine regulation of immunity. How does an acute stressor 14 days before granuloma induction advance the wound healing process in females, but not in males? One possibility is that the restraint stress procedure was experienced differently by females and males. If perceived as more traumatic, for example, memory of the first stressor might change the second experimental procedure, namely induction of the granuloma. Another hypothesis is that stressed females may activate another system shown to have immunoenhancing properties. The female response to stress in some species has been colloquially characterized as “tend-and-befriend” (56), rather than “fight or flight,” largely based on evidence that this gender’s requirements for raising offspring necessitated development of another stress regulatory system. For example, oxytocin, a hormone secreted by females in social contexts, may function as part of an alternative stress regulatory system that facilitates wound healing (17).

On the basis of the social ecology of the Norway rat, we hypothesized that there would be selective pressure for sex differences in links between social interactions, stress, and immune function. The survival and reproductive success of male Norway rats, which live naturally during mating seasons as social isolates (2), may depend on physiological mechanisms and health trajectories that are less enmeshed in the group context than females. Differences in the interaction of stress and the inflammatory response may well arise from dissimilarities in the biobehavioral costs of social contexts for males and females.

In this series of experiments, we developed a model system uniquely relevant to findings of increased morbidity among isolated and lonely individuals (4, 5) and to the study of sex differences in health across socially disparate communities. The observed sex differences in response to stress also reveal that the biological consequences of social isolation are not the same as those of an acute stressor. Social isolation suppressed the inflammatory response of males and females, whereas the acute stressor had a sexually dimorphic effect, enhancing the inflammatory response in females and delaying it in males. This mechanism may, in part, explain the observation that, in humans, men with low levels of social integration are more vulnerable to disease and death than women (28). The outcome of these animal studies may ultimately lead to a mechanism, at

the intersection of stress and immunity, which accounts for greater vulnerability to disease and death among men who are lonely or bereaved and the corresponding resilience in women.

ACKNOWLEDGMENTS

We thank John Sheridan and Esther Sternberg for formative discussions and Brian Prendergast for invaluable editorial assistance.

GRANTS

This work was supported by National Institutes of Health Merit Award R37-MH-41788, a grant from the Mind-Body Network of the John D. and Catherine T. MacArthur Foundation (to Martha K. McClintock), National Institute of Aging Program Project Grant P01-AG-18911, and National Institute of Environmental Health Sciences Grant P50-ES-012382-02 (awarded to the Center for Interdisciplinary Health Disparities Research).

REFERENCES

1. Baldwin DR, Wilcox ZC, and Bayliss RC. Impact of differential housing on humoral immunity following exposure to an acute stressor in rats. *Physiol Behav* 57: 649–653, 1995.
2. Barnett SA. *The Rat*. Chicago: University of Chicago Press, 1963.
3. Benton D and Brain PF. Behavioral and adrenocortical reactivity in female mice following individual or group housing. *Dev Psychobiol* 14: 101–107, 1981.
4. Berkman L and Syme SL. Social networks, host resistance, and mortality: a nine-year follow-up study of Alameda County residents. *Epidemiology* 109: 186–204, 1979.
5. Berkman LF. The role of social relations in health promotion. *Psychosom Med* 57: 245–254, 1995.
6. Brown JR and DuBois RN. Cyclooxygenase as a target in lung cancer. *Clin Cancer Res* 10: 4266s–4269s, 2004.
7. Cacioppo JT. Lonely traits and concomitant physiological processes: the MacArthur social neuroscience studies. *Int J Psychophysiol* 35: 143–154, 2000.
8. Cacioppo JT, Hawkey LC, Crawford LE, Ernst JM, Burleson MH, Kowleski RB, Malarkey WB, Van Cauter E, and Bertson GG. Loneliness and health: potential mechanisms. *Psychosom Med* 64: 407–417, 2002.
9. Chover-Gonzalez A, Jessop DS, Tejedor-Real P, Gibert-Rahola J, and Harbuz MS. Onset and severity of inflammation in rats exposed to the learned helplessness paradigm. *Rheumatology* 39: 764–771, 2000.
10. Chrousos GP. Seminars in medicine of the Beth Israel Hospital, Boston: the hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med* 332: 1351–1362, 1995.
11. Cobb S. Social support as a moderator of life stress. *Psychosom Med* 332: 300–314, 1976.
12. Cohen S. Psychosocial models of the role of social support in the etiology of physical disease. *Health Psychol* 7: 269–297, 1988.
13. Da Silva JA. Sex hormones and glucocorticoids: interactions with the immune system. *Ann NY Acad Sci* 876: 102–117, 1999.
14. Da Silva JA. Sex hormones, glucocorticoids, and autoimmunity: facts and hypotheses. *Ann Rheum Dis* 54: 6–16, 1995.
15. Dantzer R, Wollman EE, Vitkovic L, and Yirmiya R. Cytokines, stress, and depression. Conclusions and perspectives. *Adv Exp Med Biol* 461: 317–329, 1999.
16. Deschamps S, Woodside B, and Walker CD. Pups presence eliminates the stress hyporesponsiveness of early lactating females to a psychological stress representing a threat to the pups. *J Neuroendocrinol* 15: 486–497, 2003.
17. Detillion CE, Craft TK, Glasper ER, Prendergast BJ, and DeVries AC. Social facilitation of wound healing. *Psychoneuroendocrinology* 8: 1004–1011, 2004.
18. Dhabhar FS. Stress-induced enhancement of cell-mediated immunity. *Ann NY Acad Sci* 840: 359–372, 1998.
19. Dhabhar FS and McEwen BS. Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: a potential role for leukocyte trafficking. *Brain Behav Immun* 11: 286–306, 1997.
20. Dhabhar FS and McEwen BS. Enhancing versus suppressive effects of stress hormones on skin immune function. *Proc Natl Acad Sci USA* 96: 1059–1064, 1999.
21. Dhabhar FS, Satoskar AR, Bluethmann H, David JR, and McEwen BS. Stress-induced enhancement of skin immune function: a role for gamma interferon. *Proc Natl Acad Sci USA* 97: 2846–2851, 2000.

22. **Gans SE and McClintock MK.** Individual differences among female rats in the timing of the preovulatory LH surge are predicted by lordosis reflex intensity. *Horm Behav* 27: 403–417, 1993.
23. **Hambleton P and Miller P.** Studies on carrageenin air pouch inflammation in the rat. *Br J Exp Pathol* 70: 425–433, 1989.
24. **Hambleton P and Miller P.** Studies on immunological air pouch inflammation in the rat. *Int Arch Allergy Immunol* 87: 70–75, 1988.
25. **House JS.** Social isolation kills, but how and why? *Psychosom Med* 63: 273–274, 2001.
26. **House JS.** Understanding social factors and inequalities in health: 20th century progress and 21st century prospects. *J Health Soc Behav* 43: 125–142, 2002.
27. **House JS, Landis KR, and Umberson D.** Social relationships and health. *Science* 241: 540–545, 1988.
28. **House JS, Robbins C, and Metzner HL.** The association of social relationships and activities with mortality: prospective evidence from the Tecumseh Community Health Study. *Am J Epidemiol* 116: 123–140, 1982.
29. **Hundal RS, Petersen KF, Mayerson AB, Randhawa PS, Inzucchi S, Shoelson SE, and Shulman GI.** Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. *J Clin Invest* 109: 1321–1326, 2002.
30. **Johnson JD, O'Connor KA, Deak T, Stark M, Watkins LR, and Maier SF.** Prior stressor exposure sensitizes LPS-induced cytokine production. *Brain Behav Immun* 16: 461–476, 2002.
31. **Johnson JD, O'Connor KA, Watkins LR, and Maier SF.** The role of IL-1 β in stress-induced sensitization of proinflammatory cytokine and corticosterone responses. *Neuroscience* 127: 569–577, 2004.
32. **Karalis KP, Kontopoulos E, Muglia LJ, and Majzoub JA.** Corticotropin-releasing hormone deficiency unmasks the proinflammatory effect of epinephrine. *Proc Natl Acad Sci USA* 96: 7093–7097, 1999.
33. **Karp J, Moynihan J, and Ader R.** Psychosocial influences on immune responses to HSV-1 infection in BALB/c mice. *Brain Behav Immun* 11: 47–62, 1997.
34. **Karp JD, Moynihan JD, and Ader R.** Effects of differential housing on the primary and secondary antibody responses of male C57BL/6 and BALB/c mice. *Brain Behav Immun* 7: 326–333, 1993.
35. **Kawai N, Tsuji S, Tsujii M, Ito T, Yasumuru M, Kakiuchi Y, Kimura A, Komori M, Sasaki Y, Hayashi N, Kawano S, DuBois R, and Hori M.** Tumor necrosis factor alpha stimulates invasion of SRC-activated intestinal cells. *Gastroenterology* 122: 331–339, 2002.
36. **Kiecolt-Glaser JK and Glaser R.** Chronic stress and mortality among older adults. *JAMA* 282: 2259–2260, 1999.
37. **Kiecolt-Glaser JK, Preacher KJ, MacCallum RC, Atkinson C, Malarkey WB, and Glaser R.** Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proc Natl Acad Sci USA* 100: 9090–9095, 2003.
38. **Lee TM and McClintock MK.** Female rats in a laboratory display seasonal variation in fecundity. *J Reprod Fertil* 76: 51–59, 1986.
39. **LeFevre J and McClintock MK.** Reproductive senescence in female rats: a longitudinal study of individual differences in estrous cycles and behavior. *Biol Reprod* 38: 780–789, 1988.
40. **Maes M, Bosmans E, De Jongh R, Kenis G, Vandoolaeghe E, and Neels H.** Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. *Cytokine* 9: 853–858, 1997.
41. **Marucha PT, Kiecolt-Glaser JK, and Favagehi M.** Mucosal wound healing is impaired by examination stress. *Psychosom Med* 60: 362–365, 1998.
42. **Misiewicz B, Griebler C, Gomez M, Raybourne RB, Zelazowska E, Gold PW, and Sternberg EM.** The estrogen antagonist tamoxifen inhibits carrageenin induced inflammation in LEW/N female rats. *Life Sci* 58: 281–286, 1996.
43. **Misiewicz B, Zelazowska E, Raybourne RB, Cizza G, and Sternberg EM.** Inflammatory responses to carrageenin injection in LEW/N and F344/N rats: LEW/N rats show sex- and age-dependent changes in inflammatory reactions. *Neuroimmunomodulation* 3: 93–101, 1996.
44. **Monje M, Toda H, and Palmer TD.** Inflammatory blockade restores adult hippocampal neurogenesis. *Science* 302: 1760–1764, 2003.
45. **Murray HW.** Granulomatous inflammation: host antimicrobial defense in the tissues in visceral leishmaniasis. In: *Inflammation: Basic Principles and Clinical Correlates* (3rd ed.), 1999, p. 977–994.
46. **Ohuchi K, Sato H, Komabayashi T, Tsurufuji S, Satoh H, and Levine L.** Prostaglandin production by minced carrageenin granuloma tissue of rats and its inhibition by dexamethasone and cycloheximide. *Prostaglandins Med* 4: 267–274, 1980.
47. **Ridker PM.** High sensitivity C-reactive protein, inflammation, and cardiovascular risk: from concept to clinical practice to clinical benefit. *Am Heart J* 148: S19–S26, 2004.
48. **Rohleder N, Schommer NC, Hellhammer DH, Engel R, and Kirschbaum C.** Sex differences in glucocorticoid sensitivity of proinflammatory cytokine production after psychosocial stress. *Psychosom Med* 63: 966–972, 2001.
49. **Seeman TE, Kaplan GA, Knudsen L, Cohen R, and Guralnik JM.** Social network ties and mortality among the elderly in the Alameda County Study. *Am J Epidemiol* 126: 714–723, 1987.
50. **Seeman TE, Singer BH, and Charpentier P.** Gender differences in patterns of HPA axis response to challenge: MacArthur studies of successful aging. *Psychoneuroendocrinology* 20: 711–725, 1995.
51. **Selye H.** On the mechanism through which hydrocortisone affects the resistance of tissues to injury: an experimental study with the granuloma pouch technique. *J Am Med Assoc* 152: 1207–1213, 1953.
52. **Sheridan JF.** Stress-induced modulation on anti-viral immunity. *Brain Behav Immun* 12: 1–6, 1998.
53. **Soilleux E.** *What is a Granuloma?* Cambridge, UK: University of Cambridge, 2000.
54. **Stephoe A, Owen N, Kunz-Ebrecht SR, and Brydon L.** Loneliness and neuroendocrine, cardiovascular, and inflammatory stress responses in middle-aged men and women. *Psychoneuroendocrinology* 29: 593–611, 2004.
55. **Takahashi LK and Lore RK.** Intermale and maternal aggression in adult rats tested at different ages. *Physiol Behav* 29: 1013–1018, 1982.
56. **Taylor SE, Klein LC, Lewis BP, Gruenewald TL, Gurung RA, and Updegraff JA.** Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. *Psychol Rev* 107: 411–429, 2000.
57. **Xie Z, Smith CJ, and Van Eldik LJ.** Activated glia induce neuron death via MAP kinase signaling pathways involving JNK and p38. *Glia* 45: 170–179, 2004.