LIPPINCOTT WILLIAMS & WILKINS

PSYCHOSOMATIC MEDICINE

Volume 59(3) May/June 1997 pp 213-221

Chronic Social Stress, Social Status, and Susceptibility to Upper Respiratory Infections in Nonhuman Primates

[Original Article]

Cohen, Sheldon PhD; Line, Scott DVM; Manuck, Stephen B. PhD; Rabin, Bruce S. MD, PhD; Heise, Eugene R. PhD; Kaplan, Jay R. PhD

From the Department of Psychology, Carnegie Mellon University (S.C.), Departments of Comparative Medicine (S.L., J.R.K.) and Microbiology and Immunology (E.R.H.), Bowman Gray School of Medicine, Behavioral Physiology Laboratory, Department of Psychology (S.B.M.), University of Pittsburgh, and Department of Pathology (B.S.R.), University of Pittsburgh School of Medicine.

Address reprint requests to: Sheldon Cohen, PhD, Department of Psychology, Carnegie Mellon University, Pittsburgh, PA 15213. Address e-mail to SCOHEN@cmu.edu.

Received for publication January 15, 1996; revision received June 20, 1996.

Outline

Graphics

Abstract

Objective: The objective of the study was to assess the roles of social stress and social status in susceptibility to upper respiratory infection. Method: Sixty male cynomolgus monkeys were randomly assigned to stable or unstable social conditions for 15 months. Two markers of social status, social rank and percent of behaviors that were submissive, were assessed at independent observation periods. Endocrine, immune, and behavioral responses were each assessed (at 3-month intervals) during the 9th through 14th months of the study. At the beginning of the 15th month, all animals were exposed to a virus (adenovirus) that causes a common-cold-like illness. The primary outcome was whether or not an animal developed an infection (shed virus) after viral exposure. Results: Although the social instability manipulation was associated with increased agonistic behavior as indicated by minor injuries and elevated norepinephrine responses to social reorganizations, the manipulation did not influence the probability of being infected by the virus. However, low social status (as assessed by either marker) was associated with a substantially greater probability of being infected. It was also associated with less body weight, greater elevated cortisol responses to social reorganizations, and less aggressive behavior. However, none of these characteristics could account for the relation between social status and infection. Conclusions: Social stress was not associated with susceptibility to infection. However, animals with lower social status were at higher risk than high social status animals.

Key words: stress, dominance, infectious disease, immune function, endocrine.

INTRODUCTION

There is a substantial core of correlational evidence from studies of humans that chronic social stressors are associated with increased risk for upper respiratory illnesses [1]. This includes prospective studies of naturally occurring infections in community samples (eg, [2-4]) as well as viral-challenge trials where psychological assessments are followed by an intentional exposure to an upper respiratory virus [5-7]. Because this work is correlational, it is always possible that these associations may be attributable to third (spurious) factors such as personality characteristics that contribute to both stress and illness.

Experimental studies of the effects of prolonged stressful events on susceptibility to infection offer the solution to this problem. However, it is neither ethical nor feasible to randomly assign human subjects to chronically stressful experiences. Consequently, we conducted an experimental study of the effects of a prolonged social stressor on disease susceptibility in nonhuman primates. Earlier work suggested that monkeys assigned to unstable social groups demonstrated a suppression of cellular immune function as assessed by mitogen-stimulated lymphocyte proliferation [8]. This is in addition to other research with monkeys demonstrating the sensitivity of their immune systems to social manipulation (eg, [9-11]). In the study presented here, we asked whether unstable social environments influence the probability of developing infection (virus replicates in host) after viral exposure. The single human study addressing the

relation of stress and infection suggests that although greater perceptions of stress and negative affect are associated with greater risk for infection, the occurrence of stressful events themselves are not [6].

Social status has been established as a primary dimension of behavioral variability among individuals in many primate species. Status is also associated with biological correlates thought to have implications for susceptibility to infectious agents [13,14]. Operationally, social dominance denotes an animal's ability to defeat others in antagonistic encounters [15]. Among groups of animals it is possible to generate hierarchies of relative social dominance in which individual monkeys are ranked by the number of other animals habitually defeated in fight interactions (ie, ranked from most dominant to most subordinate). Consistent with their subordinance, lower ranked animals demonstrate more submissive and fewer aggressive acts than their more highly ranked counterparts (eg, [16]). It is widely hypothesized that subordination is associated with adverse health outcomes including susceptibility to infectious disease [15,16]. Lower social rankings have been associated with biological markers thought to influence infectious disease susceptibility such as elevated basal adrenocorticotropic hormone (ACTH) [17], elevated resting cortisol [13], and larger adrenal weights [14]. Although primate studies have not directly assessed the role of dominance in infectious susceptibility, a study in which pigs were inoculated with Aujeszky disease virus found that those of lower social status had higher levels of both morbidity and mortality [18].

Human studies of socioeconomic status (SES) and health have consistently demonstrated that lower SES is associated with poorer health (see [19-21]). Although several unique (to the human condition) mediators have been hypothesized to explain these relations (eg, access to medical care and variations in the physical characteristics of living environments), the psychological concomitants of social rank still remain a candidate. In unpublished data from our earlier study [8], social rank was not associated with a marker of cellular immune response (mitogen-stimulated lymphocyte proliferation) in primates. However, there is reason to think that the endocrine alterations associated with social rank play a role in infectious susceptibility; hence, we pursued the role of dominance as a predictor of susceptibility to infection in the current work.

METHODS

The subjects were 60 healthy feral adult male, cynomolgus monkeys (Macaca fascicularis) with an average age of approximately 7 years. Animals were randomly assigned to stable (N = 30) or unstable (N = 30) social conditions for a 15-month period. During the last week of each month they were observed to determine dominance rank in their group. Additional behavioral (submission, affiliation, and aggression), endocrine (epinephrine, norepinephrine, and cortisol), and immune (enumeration of white blood cells and mitogen-stimulated lymphocyte proliferation) data were also collected over the course of the study. At the beginning of the 15th month, all animals were inoculated with an adenovirus that causes a common-cold-like illness. The dependent variable was viral infection as assessed by isolation of the virus in nasal secretions 2 and 4 days after inoculation.

In both experimental conditions the monkeys were housed in groups of five. In the unstable condition, memberships were redistributed (reorganized) on a monthly basis so that in each month every monkey was housed with three or four monkeys who were not in its previous group. In the socially stable condition, the animals remained in the same social group for the 15-month period. This redistribution schedule results in persistent social disruption and agitation, yet it is similar to the exposure of males to social strangers that occurs frequently in the wild [14,22,23].

Multiple reorganizations contribute to suppression of cellular immune response [8] and to the development of coronary artery disease [14].

After the first 8 months, the animals were exposed to an influenza virus that resulted in infection of all animals. The data reported in this study focus on the following 7 months culminating in exposure to an adenovirus. This virus was chosen because a) we expected the adenovirus to infect between 20% and 40% of the monkeys, allowing us to assess susceptibility to infection, and b) exposure to the Singapore B influenza virus used in the first viral challenge does not provide immunity to this virus.

The animals were fed ad libitum (always food left over) and consumed approximately 150 calories per kilogram of body weight per day. The tips of the canine teeth of all animals were clipped to minimize injuries. Monkeys were observed by a behavioral technician every day. Those observed to be lethargic, withdrawn, or depressed were immediately given veterinary care. Animals were also weighed at least once a month with those exhibiting any weight loss similarly examined. Incidences of injuries were recorded.

Blood samples and nasal washes were collected from anesthetized (ketamine HCl at 15 mg/kg) monkeys. The monkeys were trained for handling with techniques developed in a previous series of studies [10]. The rapidity and ease with which the sampling can be done serves to ensure that samples are obtained without compromise by the stress of sampling (eg, [24]). All serum and nasal wash samples were frozen at -70degreesC. Samples collected for immunological assays requiring fresh blood lymphocytes were processed immediately on site.

Blood samples were always drawn over a 1-hour period that began between 8:00 AM and 9:30 AM. Technicians entered the building and immediately began anesthetizing and bleeding one half of the animals (N = 30). These animals were drawn equivalently from each experimental condition. Animals were housed five to a pen, and an entire pen was let into a catch cage where they were anesthetized. No more than 10 to 12 minutes elapsed between capture of a pen and blood sampling. Sampling within each pen was random. The entire set of 30 animals was bled within 1 hour. The remaining animals (also drawn equivalently from each experimental condition) were bled at the same time of day, but on the day after the first sample.

All procedures involving animals were conducted in compliance with state and federal laws, standards of the U. S. Department of Health and Human Services, and guidelines established by our Institutional Animal Care and Use Committee.

Social Dominance

For cynomolgus monkeys, a series of specific facial expressions, postures, and vocalizations indicate the occurrence of a fight, and an animal's relative social status may be based on these. Typically, one animal in a fight signals aggression and the other signals submission [23]. This highly asymmetric pattern allows fight outcomes to be judged in terms of clear winners and losers (eg, [15]). The animal in each group that defeats all others (as evidenced by his ability to elicit consistently submissive responses) is designated as the first-ranking monkey. The one that defeats all but the first-ranking monkey is designated as the second-ranking monkey, and so forth. In general, dominance relationships within small groups are transitive; that is, if Monkey 1 is dominant to Monkey 2 and Monkey 2 is dominant to Monkey 3, then Monkey 1 is dominant to Monkey 3 also [23]. Formal rankings were made on the basis of ad libitum observations of each group during the last week of each reorganization period (or the last week of the month for

animals in stable social groups). Technicians recorded all fights observed during a 30-minute period. Each animal was assigned a rank for each month based on the outcomes of these fights. Informal daily rankings by technicians during the first 4 months of the study indicated extreme stability of ranks within the 1-month reorganization periods in both stable and unstable animals.

Social status was also relatively stable over the course of the study (months 1-14). This is consistent with earlier studies with this paradigm [25] and is thought to reflect the role of underlying temperamental characteristics (such as aggressiveness and fearfulness) that favor certain animals becoming dominant and others subordinate in this controlled and structured social situation. Ranks aggregated over the period of the first study (months 1-8) correlated.97 ($p < .001$) with ranks aggregated over the period of the current study (months 9-14). Because they represent the more proximal assessments of social rank, the primary operational definition of social rank in this article is average rank aggregated over months 9-14. However, use of a social rank measure based on all 14 months results in identical conclusions. In a secondary analysis we also use a rank measure based solely on rank as assessed during the 14th month of the study-the ranking just preceding exposure to the virus.

Social Behaviors

Behavioral data were collected by scan (snapshot) sampling where the technician notes the behavior of each animal at set intervals [26]. This technique allows the time budgets of animals to be quantified in terms of the percentage of time spent in various activities. Three trained observers were used with interobserver reliabilities over 90%. In this study, the behavior of each animal was noted (scanned) every 45 seconds during 30-minute observation periods. Consequently each animal had 40 scans per observation session. Observation sessions were scheduled twice a day (once in the morning and once in the afternoon). Data were collected on the 8th, 10th, and 15th days after the beginning of months 11 and 14. These observations served as indicators of social behaviors during the study but before viral inoculation. Behavior was recorded in terms of 32 individual aggressive, affiliative, and submissive motor patterns [14].

Because social rank is defined in terms of submissive behaviors, these two concepts are similar (although based on independent observations). As expected, percent of the animals' time involved in submissive behaviors correlated ($r = .77$, $p < .001$) with mean social rank.

Neuroendocrine Assessments

Blood samples were collected just before and just after the ninth and twelfth reorganizations for the assessment of serum cortisol. Samples were collected just before and just after the twelfth reorganizations for the assessment of platelet catecholamines. (For simplicity sake, we use the term reorganization in this article to refer to the point in time (approximately monthly) when animals in the unstable group were reorganized, although there was no reorganization in the stable group.)

Platelet Catecholamines. We measured platelet catecholamines as an indicator of chronic sympathetic activation. Platelets contain both epinephrine (epi) and norepinephrine (norepi) in concentrations proportional to basal plasma levels, as the uptake and release in platelets occur at a slower rate [27,28].

Six milliliters of blood was collected into ethylenediaminetetraacetic acid (EDTA) Vacutainer tubes and placed on ice. Sodium metabisulfite was added and the blood was centrifuged at 200g

at 4degreesC for 15 minutes; the platelet rich plasma (PRP) was transferred to a polypropylene tube, and centrifuged for 40 minutes at 3000g. The plasma layer was removed and the pellet frozen at -70degreesC. For the extraction of catecholamines the platelet pellet was thawed to 4degreesC and 1.1 ml of 0.1 N perchloric acid (PCA) added with 500 pg of 3,4-dihydroxybenzylamine in 0.1 N PCA. The suspension was homogenized for 30 seconds in a tissue homogenizer and the homogenate centrifuged at 3000g for 20 minutes at 4degreesC. Then 1.0 ml of the supernatant was removed and added to 20 mg of Alumina and 500 microl 1 M Tris-HCl buffer (pH 8.6, at 20degreesC) containing 0.2 M disodium EDTA and 3 mM $Na_2 S_2 O_5$ (sodium metabisulfite), followed by shaking for 15 minutes and centrifugation at 2000g for 1 minute. The pellet was washed twice in 1.0 ml of 16.5 mM Tris-HCl buffer (pH 8.6, 20degreesC) containing 10 mM EDTA and 1 mM $Na_2 S_2 O_5$ and centrifuged as above. The pellet was suspended in 100 microl 0.1 N PCA, centrifuged at 2000g at 4degreesC for 1 minute, and the supernatant collected and filtered through a 0.22 microm filter for HPLC (high pressure liquid chromatography) analysis and protein determination.

Samples were analyzed by HPLC with electrochemical detection (Waters 460 ED) at +0.65 mV potential. Both epi and norepi are expressed as picograms per milligram of protein.

Serum Cortisol. Cortisol was measured by a radioimmunoassay (RIA; [29]). Serum cortisol was extracted with diethylether. Aliquots were incubated with^3 H-cortisol (tracer) and an antibody to cortisol, antisera F3-314 from Endocrine Sciences. The reaction was stopped with charcoal dextran buffer pH 7, the sample centrifuged, and the aqueous portion counted in a scintillation counter. The intraassay coefficient of variation was < 5% and the interassay coefficient was 5.4%.

Immune Assessments

Blood samples were collected just before and just after the eleventh and fourteenth reorganizations for the immune assessments.

Complete Blood Count (CBC) and Differential. A CBC and differential were obtained from a 0.5-ml sample of blood collected with EDTA.

Lymphocyte Subsets. Circulating populations of T-cell subsets, B cells, and NK cells were assessed in whole blood using dual color fluorescence analysis with a FACScan flow cytometer. Lymphocyte subsets were analyzed using Becton-Dickson monoclonal antibodies labeled with either fluorescein (FITC) or phycoerythrin (PE) to quantify $CD20^+$ (B), and $CD2^- CD16^+$ (NK) cells and Exalpha Corporation antibodies to quantify $CD4^+$ (helper T), $CD8^+$ (suppressor/cytotoxic T), and CD2 (+) (T). These mouse antihuman antibodies cross-react with cynomolgus macaque lymphocytes [30,31]. Isotype controls labeled with FITC or PE were used to assess nonspecific binding.

Mitogen Stimulation. A whole blood assay was conducted to establish a dose response curve for phytohemagglutinin (PHA) at final concentrations of 5.0, 10.0, and 20.0 microg/ml and for concanavalin A (ConA) at concentrations of 5.0, 10.0, 20.0, and 30.0 microg/ml. Blood was diluted 1:10 with RPMI-1640 tissue culture medium, supplemented with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), 2 mM glutamine, and 50 microg gentamicin per milliliter. One hundred microliters of diluted whole blood was added to a 96-well, flat-bottomed culture plate containing 100 microl of ConA or PHA solution prepared in RPMI in one of the final concentrations. Background proliferation was measured by incubating

cells in RPMI only. Each assay was performed in triplicate. Plates were incubated for 96 hours at 37degreesC in air and 5% CO_2. Twenty-four hours before being harvested for counting, the wells were pulsed with 1 microCi of tritiated thymidine. The difference in counts per minute between stimulated and unstimulated samples was determined separately for each concentration. The log of difference in counts per minute between stimulated and unstimulated samples from the optimal concentration (the concentration resulting in the highest mean count across animals) was used in the analyses [32]. The optimal concentrations were 20 microg/ml for PHA and 30 microg/ml for ConA.

Viral Challenge

We used primate adenovirus SV17 supplied by the Virus Reference Laboratory, San Antonio, Texas. None of the monkeys had serum immunoglobulin G (IgG) antibody to this virus before the study. The virus was purified and adjusted to a concentration of 1.0×10^6 $TCID_{50}$ /ml (TCID = tissue culture infectious dose). It was diluted 1:100 to prepare a final challenge dose of 1.0 ml per monkey. Five days after the 15th reorganization, each subject was sedated with 15 mg/kg ketamine HCl and given 0.5 ml of the viral suspension into each nostril.

Verifying Infection

Nasal secretion samples were collected on the day of inoculation (control) as well as two and four days afterward. Two and four days were chosen because they are within the incubation window for the virus but soon enough after the inoculation to minimize the possibility of animal-to-animal transmission of the infectious agent. Nasal washes were used to obtain secretion samples for the viral isolation assay. After sedation with ketamine HCl (15 mg/kg intramuscularly), 3 ml of phosphate buffered saline was infused into each nostril. At the same time the saline was suctioned from the back of the throat. Samples were collected into sterile centrifuge tubes and frozen at -70degreesC until the viral isolation assay was conducted.

Viral Isolation Assay. All samples were centrifuged to remove particulate matter, then inoculated into Vero cells grown in 24-well culture plates in Eagle's Minimal Essential Medium containing gentamicin, amphotericin B, and fetal bovine serum. Inoculated cultures were observed daily for 10 days for evidence of viral cytopathology. Cultures positive after two passages in Vero cells were inoculated onto cell monolayers, stained with hematoxylin and eosin, and examined for adenoviral intranuclear inclusion to confirm infection. None of the control (preinoculation) samples were positive for virus. Animals with confirmed infection on either Day 2 or 4 after inoculation were considered infected. Thirteen (22%) of the animals were infected by the virus.

RESULTS

Analyses

One animal in the unstable condition died during the course of the study, hence the analyses are based on 59 animals. Unless otherwise indicated, the analytic models were multiple regressions in the case of continuous outcome variables and logistic regressions where infection is the outcome. In the primary analyses, stability was entered as a dichotomous variable (stable vs unstable) and average social rank as a continuous variable. Secondary analyses use categorical breakups of social rank to clarify the nature of the relation and to provide assessments of the effect size (odds ratios). Stability and social rank were entered simultaneously followed by the entry of the stability-by-rank interaction. When the F statistic for social rank was significant, we

also report the Pearson or point-biserial correlation to indicate effect direction and provide an estimate of effect size.

Viral Infection

Neither stability nor the stability-by-social rank interactions approached significance in the analyses predicting infection. The failure to find these statistical relations is consistent with a lack of mean differences between groups and hence not attributable to insufficient power (sample size). Although all analyses include stability and the interaction, for the sake of brevity, we report only associations with social rank.

Lower social status (higher average social rank number) was associated with higher rates of infection ($b = .83$, $df = 1$, $p < .01$). To obtain an estimate of effect size (odds ratio) we also broke the social ranking at the median (<3 = high social status) and compared the two groups. Among low status animals, 32% were infected, whereas among high status animals, 11% were infected (odds ratio = 3.77, $p < .07$). However, breaking the ranks into quintiles seemed to provide the most information. The percent of animals infected at each quintile is presented in Figure 1. We then fit a logistic regression model in which we contrasted each of the four lowest quintiles with the highest. Only the comparison of the lowest and highest quintiles was significant (odds ratio = 16.42, $df = 1$, $p < .03$).

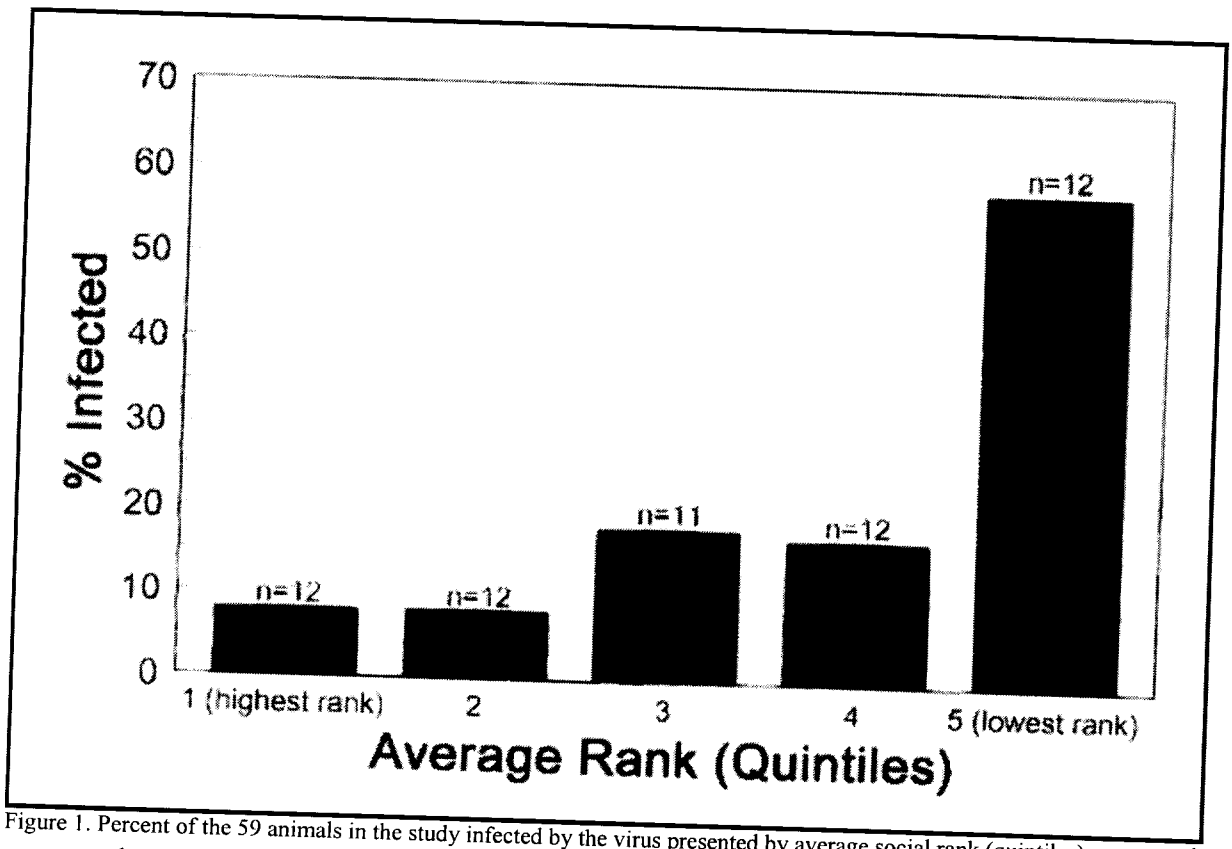

Figure 1. Percent of the 59 animals in the study infected by the virus presented by average social rank (quintiles) aggregated across months 9-15; 1 refers to the highest rank and 5 to the lowest.

We conducted the identical analyses using the social rank observed in the single month just before the viral challenge (end of the 14th month). An analysis evaluating infection rates in the

high status (Ranks 1 and 2) vs the lower status (Ranks 3, 4, and 5 animals) indicate a greater risk among those in the low status group (odds ratio = 5.10, df = 1, $p < .05$). Thirty-one percent of the low status group were infected vs 8% of the high status group. A comparison of each rank to the highest status (Rank 1) animals suggests that only the lowest status animals are significantly different (odds ratio comparing the lowest to highest = 19.76, df = 1, $p < .02$). The rates of infection starting at the highest social status were 8%, 8%, 8%, 25%, and 64%. These results support the same relation as found with average social rank across the study.

We also conducted similar analyses using the percent of all behaviors that were submissive acts as the marker of social status instead of social rank. As noted earlier, although highly intercorrelated, these two measures were assessed independently. Again stability was unrelated to infection, but higher rates of submission (lower social status) were associated with higher rates of infection (b = 73.10, df = 1, $p < .004$). We also broke percent of behaviors that were submissive at the median (< 1.25% = low submission) and found 7% of those below the median were infected vs 37% of those above the median (odds ratio = 7.54, df = 1, $p < .02$). Additional breakdowns of these data (eg, quintiles) did not provide any further clarification of the nature of the effect. In the remainder of this article, we use average social rank as the marker of social status. However, analyses using rate of submissive behaviors yield similar results.

Body Weight

Average body weight (across months 9 through 15 of the study) by average rank is presented in Table 1. Although social stability was not associated with body weight, higher social rank (lower social status) was associated with lower body weight ($r = -.30$, $p < .03$). Because body weight may be a marker of physical robustness, it was necessary to assess its role in the relation between social status and infection rates. A logistic regression indicated that body weight did not predict susceptibility to infection. Even so, to be conservative, we reran the analyses of infection rates adding average body weight as a covariate. Average social rank (b =.82, df = 1, $p < .02$) still predicted infection.

Average Rank	Mean[a]	SD	N
1 (highest status)	6.38	1.18	12
2	6.16	1.32	12
3	5.35	0.48	11
4	5.77	0.63	12
5 (lowest status)	5.73	0.44	12

[a] Means collapse across stable and unstable conditions.

Table 1. Average Body Weight (in Kilograms) by Average Social Rank.

Social Behavior

A series of regressions were fit to determine whether either stability or social status predicted the average percent of aggressive or affiliative behaviors assessed before viral-challenge. A square root transformation was applied to aggression to approximately normalize the distribution. Stability was not associated with either affiliation or aggression. Moreover, social

status was not associated with affiliation. On the other hand, greater status was associated with higher rates of aggressive acts [$F(1,56) = 49.57$, $p < .001$, $r = .68$]. There was no significant interaction between stability and social status. The percent of observed behaviors that were aggressive and affiliative are presented by social rank in Table 2.

Average Rank	% Aggressive Behaviors		% Affiliative Behaviors	
	Mean[a]	N	Mean	N
1 (highest status)	2.5	12	29.6	12
2	1.8	12	33.1	12
3	0.9	11	34.8	11
4	1.0	12	39.2	12
5 (lowest status)	0.2	12	30.0	12

[a] Means collapse across stable and unstable conditions.

Table 2. Percent of Behaviors That Were Aggressive and Affiliative by Average Social Rank.

Catecholamine and Cortisol Response

To distinguish between background (baseline) effects of the repeated reorganizations and the acute response to being reorganized, we separately examined baseline (pre-reorganization levels only) and stress-response (change from pre- to postreorganization). The means for all animals at baseline were 44.8 microg/dl (SD = 10.9) for serum cortisol,.86 $\log_{10}$ pg/mg protein (SD =.24) for platelet epinephrine, and 1.61 log ($_{10}$) pg/mg protein (SD =.24) for platelet norepinephrine. To control for possible differences in initial (preorganization) values, the stress-response was based on residual scores calculated from regressions predicting postscores from prescores. For cortisol, we calculated separate mean levels for each animal for both pre- and postreorganizations 9 and 12. Catecholamines were assessed before and after the 12th reorganization only. Because only unstable animals are reorganized, stress-response measures are limited to that group. Therefore, differences in stress response would be indicated by main effects in the case of stability, but in the case of social status, differences would be indicated by stability-by-social rank interactions. Specifically, relations would need to occur in the unstable (where animals are reorganized), but not the stable groups.

Stability. Stability was not associated with either baseline catecholamine or cortisol outcomes. There was also no effect of reorganizations on epinephrine or cortisol response. However, unstable animals showed an increased norepi response to the reorganization (.28 SD greater than the score predicted from baseline), whereas stable animals (who are not reorganized) showed a decrease in response (.28 SD less than the predicted score) over the same time period ($b = .28$, $F(1,51) = 4.68$, $p < .04$).

Social Status. Social rank was not associated with catecholamines assessed either at baseline or as response to the reorganization. It was similarly not associated with baseline cortisol. There was a marginal stability-by-social status interaction [$b = .59$, $F(1,55) = 2.67$, $p < .11$], with lower

social status associated with greater reorganization-elicited cortisol response among those in the unstable group [who were reorganized; in separate analysis b =.38, F(1,27) = 4.43, p <.05] but not among the stable group [who were not reorganized; in separate analysis b =.03, F(1,28) =.02, NS]. These data suggest that greater social status was associated with less cortisol response to reorganizations.

Immune Response

Neither functional (lymphocyte proliferation) measure was associated with social stability, or with social status. Social stability was associated with a lower percentage of monocytes [F(1,56) = 4.85, p <.04; mean of 1.55% for stable and 2.12% for unstable], and lower social status with a greater percent of CD20s [F(1,56) = 5.30, p <.03; r = -.29]. There was no stability-by-social status interaction indicating differential reactivity to reorganizations.

Mediators of the Social Status-Infection Relation

Finally, we were interested in examining whether social status associations with behavioral, endocrine, and immune responses were responsible for the relation between decreased status and increased infection. To be considered as a possible mediator of this relation, these measures need to be associated with both social status and with infection [33]. As reported earlier, aggression and percent CD20s were both associated with social status. To assess whether they were associated also with infection, we fit logistic regression models in which each (in its own regression) was used as predictor of infection. A similar model including only animals from the unstable condition was used to examine the relation between cortisol reactions to reorganizations and infection. The percent CD20s was not associated with infection. However, percent aggression (b = -13.3, Wald = 4.13; p <.05; r = -.27) was associated with infection with more aggression related to less infection. We entered aggression, social rank, and stability simultaneously into a regression predicting infection. The unstandardized coefficient for social rank was moderately reduced from.83 (Wald = 6.79; p <.01) without aggression in the model to.76 (Wald = 3.38; p <.07) with aggression in the model. The relatively small changes in coefficient and Wald statistics when aggression was added to the regression suggest that it was not an important mediator of the relation between social rank and increased risk of infection. (There is no statistical test of whether the change in coefficients when a mediator is added to the regression is significant or not. Changes in the regression coefficient and Wald statistic provide a rough estimate of the importance.)

Greater cortisol responses to reorganizations were also associated with higher rates of infection (b = 2.3, Wald = 4.41; p <.04). Because responses to reorganizations can occur only in the unstable condition, only those 29 animals were included in this analysis. We covaried cortisol responses to reorganizations out of the relation between social rank and infection in the 29 animals. The coefficient for social rank went from 1.75 (Wald = 4.43; p <.04) to 1.87 (Wald = 3.28, p <.07). Again, a small change in the Wald statistic suggests that cortisol reactivity to reorganization was not a major mediator of the relation between social rank and susceptibility to infection.

Injuries

We were concerned that group differences in the recent incidence of injuries (primarily cuts and scratches) could account for reported associations with susceptibility to infection. Although the causes of injuries are unknown, it is probable that many resulted from agonistic encounters.

We focused on injuries observed in the 2 months just preceding viral exposure. Over this period 7 of 59 monkeys (11.9%) exhibited injuries. Unstable animals were more likely to have an injury (21%) than stable animals (3%) (odds ratio = 7.55, $p < .07$; odds ratio = 7.34, $p < .01$ for the entire study-months 9-15). Injury rates were not associated with social status or with the stability-by-status interaction. Finally, recent injury rates were not associated with infection. Consequently, injuries do not provide an explanation for the relation between social status and infection.

DISCUSSION

We found the usual increase in physical agonistic encounters in the unstable groups as indicated by a higher rate of minor injuries. Animals in unstable social groups also displayed elevated norepi responses to group reorganizations. However, the stability manipulation was not associated with functional immune outcomes nor with susceptibility to infection. That stability was not related to mitogenstimulated lymphocyte proliferation is inconsistent with our own previous work demonstrating relative suppression of this function after 24 months of reorganizations [8]. The current study was considerably shorter (immune indicators actually measured at the 11th and 14th months), and lacked the triple measurement technique (three assessments within 3 weeks) used in our earlier work. The lack of association of stability and infection also presents some difficult problems of interpretation. To the extent we view the stability manipulation as a stressful event, the failure to find a relation between social stability and infection is consistent with evidence from the viral-inoculation studies in humans [6,7]. In this work, stressful life events were associated with the development of symptoms among infected persons, but not with the risk of infection. Only measures of perceived distress (perceived stress and negative affect) were associated with increased infection. Unfortunately, the conceptual distinction between events and distress is hard to apply to the monkeys and all we can say for sure is that animals in the unstable condition displayed both behavioral (injury) and endocrine evidence of a stressful experience, but this did not place them at higher risk for infection. Even so, it is interesting that animals in the unstable condition that responded to reorganizations with elevated cortisol levels (perceived distress?) were at higher risk for infection.

A strong relation was found between animals' social status and infectious susceptibility. Both markers of social status indicated that lower status animals were at greater risk for infection than their higher status counterparts. The social rank data suggested a graded (monotonic) relation but also clearly indicated that this effect was primarily attributable to the lowest quintile of social ranking animals (those with the lowest social status) being at substantially greater risk. The rate of submissive behavior data similarly indicated a monotonic relation but did not suggest that the relation was attributable primarily to animals with the highest rate of submissive behaviors. The submissive behavior data was, however, derived from observations in 2 months fairly far removed from the viral exposure and hence might have been a less accurate marker.

Although lower status animals also were found to have lower body weights, less aggressive behavior, greater reorganization-elicited cortisol responses, and alterations in some white blood cell populations, none of these could account for the lower status animals being at higher risk for infection. Aggressive behavior was the only variable to play even a small role in explaining the relation between social status and infectious susceptibility. What can account for the relation between social status and infection? It is possible that behavioral, endocrine, and immune explanations for the relation were not adequately tested here and are still viable. Our approach was to assume that prolonged social instability and social status would result in long-term stable

changes in behavioral, endocrine, and immune responses, and that sampling twice late (second stage) in the study would provide a marker of these effects. Overall, this was not an enormously effective approach. It is possible that drawing more samples (and hence greater measurement reliability) at assessment points occurring more closely to the viral inoculation would have been more effective. It is also possible that the endocrine and immune measures we used were not the most appropriate. Other endocrine (eg, testosterone) and immune (eg, production of cytokines) measures may have been more useful in this context. Unfortunately, because there are no data on how baseline levels of immune function influence infectious susceptibility in healthy animals, there is little but general conceptual ideas about the immune system to guide such choices (eg, [1]).

It is also of interest that a higher percent of aggressive behavior was also associated with less infection. (This relation was not, however, independent of social status.) That aggressive animals are less susceptible to infection could be viewed as inconsistent with commonly held beliefs about aggression as an activator of the sympathetic nervous system and hypothalamic pituitary adrenal cortical axis. It is particularly interesting that aggression and submission, two behaviors that account for a very low percent of total animal behavior (1.3% and 1.7%, respectively, in the current study), are prospectively associated with infectious susceptibility. This is contrary to the hypothesis that behavioral styles (eg, anger/hostility) act on disease susceptibility through their repeated impact on the hormonal milieu and suggest instead that they may be markers for broader behavioral or biological processes.

In short, there is a substantial prospective relation established here between a behavioral variable (social status) and infectious susceptibility. Although many (but not all) of the behavioral and biological concomitants usually associated with social status were found here, none was able to explain why this relationship exists.

Finally, we have presented the monkey model as a technique to clarify issues about the relation between psychosocial factors and infectious susceptibility in humans. We recognize, of course, that monkeys are not humans and that generalization should be made cautiously. However, macaques are old world monkeys with many morphologic, behavioral, and physiologic similarities to humans that suggest their appropriateness as a model for understanding psychosocial influences on human response to upper respiratory viral infections.

This research was supported by Grants MH47234 and RSDA MH 00721. We thank Elizabeth Nicks for assisting in data collection, Cynthia Kuhn for conducting the catecholamine assays, Janet Schlarb for assisting in data analysis, and the John D. and Catherine T. MacArthur Foundation SES and Health Planning Initiative for providing intellectual stimulation.

REFERENCES

1. Cohen S, Williamson G: Stress and infectious disease in humans. Psychol Bull 109:5-24, 1991 [Context Link]

2. Clover RD, Abell T, Becker LA, et al.: Family functioning and stress as predictors of influenza B infection. J Fam Pract 28:535-539, 1989 **Library Holdings Bibliographic Links** [Context Link]

3. Meyer RJ, Haggerty RJ: Streptococcal infections in families. Pediatrics 29:539-549, 1962 [Context Link]

4. Graham NMH, Douglas RB, Ryan P: Stress and acute respiratory infection. Am J Epidemiol 124:389-401, 1986 **Library Holdings Bibliographic Links** [Context Link]

5. Cohen S, Tyrrell DAJ, Smith AP: Psychological stress in humans and susceptibility to the common cold. N Engl J Med 325:606-612, 1991 **Library Holdings Bibliographic Links** [Context Link]

6. Cohen S, Tyrrell DAJ, Smith AP: Life events, perceived stress, negative affect and susceptibility to the common cold. J Pers Soc Psychol 64:131-140, 1993 **Ovid Full Text Library Holdings Bibliographic Links** [Context Link]

7. Stone AA, Bovbjerg DH, Neale JM, et al: Development of common cold symptoms following experimental rhinovirus infection is related to prior stressful life events. Behav Med 8:115-120, 1992 **Library Holdings Bibliographic Links** [Context Link]

8. Cohen S, Kaplan JR, Cunnick JE, et al.: Chronic social stress, affiliation and cellular immune response in nonhuman primates. Psychol Sci 3:301-304, 1992 **Library Holdings Bibliographic Links** [Context Link]

9. Coe CL: Psychosocial factors and immunity in nonhuman primates: A review. Psychosom Med 55:298-308, 1993 **Library Holdings Bibliographic Links** [Context Link]

10. Kaplan JR, Manuck SB, Heise EB, et al.: The relationship of agonistic and affiliative behavior patterns to cellular-mediated immune function among cynomolgus monkeys (Macaca fascicularis) living in unstable social groups. Am J Primatol 25:157-173, 1991 **Library Holdings Bibliographic Links** [Context Link]

11. Laudenslager M, Capitanio JP, Reite M: Possible effects of early separation experiences on subsequent immune function in adult macaque monkeys. Am J Psychiatry 142:862-684, 1985 **Library Holdings Bibliographic Links** [Context Link]

12. Sapolsky RM: Hypercortisolism among socially subordinate wild baboons originates at the CNS level. Arch Gen Psychiatry 46:1047-1051, 1989 **Library Holdings Bibliographic Links**

13. Shively C, Kaplan J: Effects of social factors on adrenal weight and related physiology of Macaca fascicularis. Physiol Behav 33:777-782, 1984 **Full Text Library Holdings Bibliographic Links** [Context Link]

14. Kaplan JR, Manuck SB, Clarkson TB, et al.: Social status, environment and atherosclerosis in cynomolgus monkeys. Arteriosclerosis 3:359-368, 1982 [Context Link]

15. Sade DS: Determinants of dominance in a group of free ranging rhesus monkeys. In Altmann S (ed), Social Communication Among Primates. Chicago, University of Chicago Press, 1967, 99-114 [Context Link]

16. Sapolsky RM: Why zebras don't get ulcers: A guide to stress, stress-related diseases, and coping New York, W. H. Freeman and Co., 1994 [Context Link]

17. Suomi SJ, Scanlan JM, Rasmussen KLR, et al.: Pituitaryadrenal response to capture in Cayo Santiago-derived group M resus monkeys. P R Health Sci J 8:171-176, 1989 **Library Holdings Bibliographic Links** [Context Link]

18. Hessing MJC, Scheepens CJM, Schouten WGP, et al.: Social rank and disease susceptibility in pigs. Vet Immunol Immunopathol 43:373-387, 1994 **Library Holdings Bibliographic Links** [Context Link]

19. Adler NE, Boyce T, Chesney MA, et al: Socioeconomic status and health: The challenge of the gradient. Am Psychol 49:15-24, 1994 **Ovid Full Text Library Holdings Bibliographic Links** [Context Link]

20. Anderson NB, Armstead CA: Toward understanding the association of socioeconomic status and health: A new challenge for the biopsychosocial approach. Psychosom Med 57:213-225, 1995 **Ovid Full Text Library Holdings Bibliographic Links** [Context Link]

21. Haan MN, Kaplan GA, Syme SL: Socioeconomic status and health: Old observations and new thoughts. In Bunker J, Gomby D, Kehrer B (eds), Pathways to Health: The Role of Social Factors. Menlo Park, CA, Henry H. Kaiser Family Foundation, 1989, 76-135 [Context Link]

22. Fleagle JG: Primate Adaptation and Evolution. New York, Academic Press, 1988 [Context Link]

23. Kaplan JR, Adams MR, Clarkson TB, Koritnik DR: Psychosocial influences on female "protection" among

cynomolgus macaques. Atherosclerosis 53:283-295, 1984 **Library Holdings Bibliographic Links** [Context Link]

24. Walker ML, Gordon TP, Wilson ME: Menstrual cycle characteristics of seasonally breeding rhesus monkeys. Biol Reprod 29:841-848, 1983 **Library Holdings Bibliographic Links** [Context Link]

25. Kaplan, J: Inhibition of coronary arthrosclerosis by propranolol in behaviorally predisposed monkeys fed an atherogenic diet. Circulation 76:1364-1372, 1987 [Context Link]

26. Altmann J: Observational study of behavior: Sampling methods. Behaviour 48:1-41, 1974 [Context Link]

27. Rosen GS, Sanfield JA, Morrow LA, Zwelfler A: Relationship between plasma and platelet epinephrine concentrations in humans. Am J Physiol 252:E334-E339, 1987 **Library Holdings Bibliographic Links** [Context Link]

28. Born GVR, Smith JB: Uptake, metabolism and release of H^3 -adrenaline by human platelets. Br J Pharmacol 39:765-778, 1970 **Library Holdings Bibliographic Links** [Context Link]

29. Plant TM, Zorub DS: A study of the role of the adrenal glands in the initiation of the hiatus in gonadotropin secretion during prepubertal development in the male rhesus monkey (Macaca mulatta). Endocrinology 114:560, 1984 **Library Holdings Bibliographic Links** [Context Link]

30. Ahmed-Ansari, A, Brodie AR, Fultz, PN, et al.: Flow microfluorometric analysis of peripheral blood mononuclear cells from nonhuman primates: Correlation of phenotype with immune function. Am J Primatol 17:107-131, 1989 **Library Holdings Bibliographic Links** [Context Link]

31. Yamada, YK, Yabe, M, Tatsume, M: Phenotypic characterization of cynomolgus monkey natural killer cells. Cell Immunol 122:524-533, 1989 **Library Holdings Bibliographic Links** [Context Link]

32. Herbert TB, Coriell M, Cohen S: Analysis of lymphocyte proliferation data: Do different approaches yield the same results? Brain Behav Immun 8:153-162, 1994 **Library Holdings Bibliographic Links** [Context Link]

33. Baron RM, Kenny DA: The moderator-mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. J Pers Soc Psychol 51:1173-1182, 1986 **Library Holdings Bibliographic Links** [Context Link]

Accession Number: 00006842-199705000-00001