

Research Report

CHRONIC SOCIAL STRESS, AFFILIATION, AND CELLULAR IMMUNE RESPONSE IN NONHUMAN PRIMATES

Sheldon Cohen,¹ Jay R. Kaplan,² Joan E. Cunnick,³ Stephen B. Manuck,⁴ and Bruce S. Rabin³
¹Carnegie Mellon University, ²Bowman Gray School of Medicine, ³University of Pittsburgh School of Medicine, and
⁴University of Pittsburgh

Abstract—We report the first experimental study of the effect of long-term (over 2 years) exposure to a stressor on cellular immune response. Forty-three male cynomolgus monkeys were randomly assigned to stable or unstable social conditions for 26 months. The proportion of time spent in affiliative behaviors was assessed by observations made twice weekly. T-cell immune response (mitogen-stimulated cell proliferation) was assessed weekly for 3 weeks immediately following the 26-month manipulation. The possibility that affiliative behavior represents an attempt to cope with social stress was supported by greater affiliation among animals in the unstable condition than in the stable condition. Animals in the unstable condition also demonstrated relatively suppressed immune response. More affiliative animals showed enhanced immune response, with the beneficial effects of affiliation occurring primarily among unstable animals. The data are interpreted as consistent with the stress-buffering hypothesis; that is, affiliation protects animals from the potentially pathogenic influences of chronic social stress.

The social environment is viewed as having both detrimental and salutary effects on health (Cassel, 1975). Changing social environments are thought of as precursors to biological changes that place persons at risk for illness (Holmes & Rahe, 1967), but strong social ties are thought to protect persons from such risks or have independent positive effects on health (Cohen, 1988).

Evidence consistent with these alternative effects of social context has been reported in studies of immune function.

Address correspondence to Sheldon Cohen, Department of Psychology, Carnegie Mellon University, Pittsburgh, PA 15213.

For example, suppression of *in vitro* cellular immunity (T-cell proliferation by mitogens) has been found among persons with disrupted social networks, including the bereaved (e.g., Schleifer, Keller, Camerino, Thornton, & Stein, 1983), people with poor marital relationships (Kiecolt-Glaser, Fisher, et al., 1987), and caretakers of Alzheimer's disease victims (Kiecolt-Glaser, Glaser, et al., 1987). Nonhuman primates show similar modulation in response to early maternal separation (Coe, Lubach, Ershler, & Klopp, 1989; Laudenslager, Capitanio, & Reite, 1985). In contrast, increased *in vitro* immune competence has been found among persons reporting strong supportive social networks (e.g., Baron, Cutrona, Hicklin, Russell, & Lubaroff, 1990) and feelings of belonging (i.e., not being lonely; Kennedy, Kiecolt-Glaser, & Glaser, 1990) and among nonhuman primates with both high rates of affiliative and low rates of aggressive behaviors (Kaplan et al., 1991).

There are limits to the existing literature. First, the impact of prolonged (chronic) social disorganization on cellular immune response is not well established. Much of the existing work involves relatively acute stressors. Work with more prolonged stressors is correlational and quasi-experimental; hence, causal inference is not possible. Second, evidence for the beneficial role of a supportive social environment is based primarily on self-reports of support. Such reports may reflect dispositionally driven biases in the perception of available supports rather than objective social resources. Only a single study of nonhuman primates assessed affiliation by behavioral observation (Kaplan et al., 1991).

We report the first experimental study of the role of long-term (over 2 years) exposure to a social stressor in cellular immune response. Nonhuman primates were randomly assigned to ei-

ther unstable or stable social conditions for 26 months, and their affiliative behaviors were closely monitored. At the end of the study, T-cell immune response was assessed weekly for 3 weeks. Hence, we were able to evaluate experimentally the effects of a chronic social stressor as well as to measure affiliative behaviors objectively over the course of the stressor.

METHODS

The subjects were 43 healthy, adult male cynomolgus monkeys (*Macaca fascicularis*). They were housed in groups of 4 or 5. For 14 months preceding the study, all of the animals were housed in stable (unchanging) social groups.¹ They were then randomly assigned to stable ($N = 22$) or unstable ($N = 21$) social conditions for a 26-month period. In the unstable condition, social groups were reorganized on a monthly basis so that in each month every monkey was housed with 3 or 4 monkeys who were not in his previous group. In the socially stable condition, the animals remained in the same social group for the 26-month period. The animals remained in their last social groups for the 3 weeks of immune measurement at the end of the study. The redistribution schedule results in persistent social disruption and agitation, yet is similar to the exposure of males to social strangers that occurs

1. This work was piggybacked on a study of the influence of diet on the development of coronary heart disease. During the 14-month period prior to the onset of this study, all of the animals consumed a diet high in saturated fat and cholesterol. During the 26-month period in which social reorganization occurred, the animals consumed a diet based on the recommendations of the American Heart Association.

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frequently in the wild (Fleagle, 1988; Kaplan, Manuck, Clarkson, Lusso, & Taub, 1982; Kaplan et al., 1983). Multiple reorganizations have been found to contribute to the development of coronary artery disease (Kaplan et al., 1982, 1983).

Behavioral observations, 30 min in length, were made twice per week on each social group. Aggressive, submissive, affiliative, and nonsocial behaviors were recorded by a combination of *ad libitum* and scan sampling (Altmann, 1974). Affiliative behavior codes included (a) passive physical contact with another animal, (b) close (but not touching) contact with another animal, and (c) grooming another animal. Factor analysis of behavioral codes indicates that these three codes form a single independent factor. Affiliation was scored by summing the total percentage of time spent in affiliative acts. Animals above the median percentage (23.6%) were identified as affiliative, while those below the median were identified as nonaffiliative. Aggressive and submissive behaviors formed a second independent factor. This factor was not associated with either social condition or immune response and is not included in the current analysis.

The tips of the canine teeth of all the animals were clipped to minimize injuries, and the monkeys were observed by a behavioral technician every day. Those noted to be lethargic, withdrawn, or depressed were immediately given veterinary care. The animals were also weighed at least once a month, with those exhibiting any weight loss similarly examined. Incidence of injuries was recorded.

An intact immune system is essential for resistance to infectious disease. There are two major components to the immune system, cellular immunity and humoral immunity. The cellular immune system comprises T-lymphocytes, which depend on the thymus gland to induce their maturation from immature cells. The function of T-lymphocytes can be measured *in vitro* by incubating the cells with a substance which induces them into mitotic division. Such agents are termed mitogens, and the amount of cell division is measured by adding a radioactive nucleotide (which is incorporated into newly synthesized DNA) to the tis-

sue culture medium that the lymphocytes are incubated in. The two mitogens most commonly used are Phytohemagglutinin (PHA) and Concanavalin A (ConA). Both stimulate T-lymphocytes to divide.

In the current study, we used both PHA and ConA. Blood was drawn weekly, for 3 consecutive weeks, following the 26-month social stability manipulation so that assessments would reflect the stability and consistency of any immune change. Blood was collected into heparinized tubes and diluted 1:10 with tissue culture medium for culture. Three concentrations of each mitogen were used to provide dose response information.

Blood was cultured in the absence of added mitogen; with 0.5, 2.5, or 5.0 µg/ml of PHA; or with 2.5, 5.0, or 10.0 µg/ml of ConA. After 54 hr of culture, tritiated thymidine was added for 18 hr, after which the cells were harvested and the amount of radioactivity incorporated into newly synthesized DNA determined as counts per minute (CPM) of radioactivity.

RESULTS

One animal in the unstable social condition was dropped from the lymphocyte proliferation analyses because of an unstimulated proliferation value more than 6 standard deviations from the mean of the sample. Analyses including this animal result in identical conclusions.

Cell proliferation data expressed in CPM of radioactivity for unstimulated samples (baseline) and for each concentration of ConA and PHA are presented in Table 1. A preliminary analysis indicated that there were no statistically reliable effects of group stability or affiliation on unstimulated (baseline) proliferation. The dependent variable, lymphocyte responsiveness, was defined as the difference in CPM between stimulated and unstimulated samples, determined separately for each concentration. To obtain approximately normal distributions, the differences between stimulated and unstimulated proliferation at each concentration were subjected to logarithmic (base-10) transformation prior to statistical evaluation. The log₁₀

Table 1. Mean proliferative response expressed in counts per minute of radioactivity of unstimulated cells, ConA-stimulated cells, and PHA-stimulated cells by social stability and affiliation conditions

Mitogen and concentration	Social condition and affiliation category			
	Stable, high affiliation (n = 8)	Stable, low affiliation (n = 14)	Unstable, high affiliation (n = 13)	Unstable, low affiliation (n = 7)
None	1,778	1,349	2,154	3,325
ConA				
2.5 µg/ml	101,117 (4.94)	85,017 (4.86)	96,152 (4.88)	59,981 (4.66)
5.0 µg/ml	132,548 (5.07)	132,793 (5.07)	129,023 (5.02)	83,000 (4.86)
10.0 µg/ml	207,111 (5.26)	202,682 (5.26)	176,237 (5.17)	117,019 (5.02)
PHA				
0.5 µg/ml	139,083 (5.09)	135,056 (5.05)	121,411 (4.83)	141,664 (5.04)
2.5 µg/ml	266,249 (5.37)	260,052 (5.38)	269,446 (5.38)	223,158 (5.31)
5.0 µg/ml	279,201 (5.41)	300,635 (5.45)	291,733 (5.43)	247,315 (5.36)

Note. All means are collapsed across the three measurement periods. Numbers in parentheses are the base-10 logarithms of the difference (Δ) between stimulated and unstimulated counts per minute.

differences for each concentration are also presented in Table 1.

ConA data were analyzed in a 2 (stable, unstable) \times 2 (affiliative, nonaffiliative) \times 3 (concentrations of 2.5, 5.0, and 10.0 $\mu\text{g/ml}$) \times 3 (measures from Week 1, Week 2, and Week 3) repeated measures analysis of variance. PHA analyses were identical except that the concentrations were 0.5, 2.5, and 5.0 $\mu\text{g/ml}$. Animals in the unstable condition showed less cell proliferation in response to ConA than those in the stable condition. The means, collapsing across concentration, affiliation, and time, were 4.96 versus 5.07 \log_{10} CPM, $F(1, 38) = 7.63$, $p < .009$. A similar mean difference was found in the case of PHA, but the difference was not reliable (5.22 vs. 5.29 \log_{10} CPM; $F(1, 38) = 1.79$, $p = .19$). The lack of interactions of the social stability manipulation with either concentration or time (week of measure) indicates that the effect of social condition was relatively consistent across the 3 weeks of assessment and across mitogen concentrations. In the case of ConA, affiliative animals showed more cell proliferation than nonaffiliative animals. The means, collapsing across concentration, social condition, and time, were 5.05 versus 4.99 \log_{10} CPM, $F(1, 38) = 3.92$, $p = .055$. There were no interactions of affiliation with either concentration or time. Figure 1 depicts the log-transformed ConA data by social stability and affiliation conditions (collapsing across concentrations and times). Although the means are consistent with stress buffering (i.e., affiliation protecting animals from stress-induced immunosuppression), the stability-by-affiliation interaction was not statistically reliable ($p = .16$). There was no main effect of affiliation in the case of PHA, nor was there an interaction between social stability and affiliation.

We were concerned that group differences in the incidence of injuries (e.g., cuts) could account for reported associations with immune function. Rates of injuries were exceptionally low. There was only one injury during the 6 months prior to immune measurement, and few occurred during the entire course of the study. Moreover, injury rates were not associated with social stability, affiliation, or their interaction.

Finally, in order to determine whether affiliative behavior was a response to the

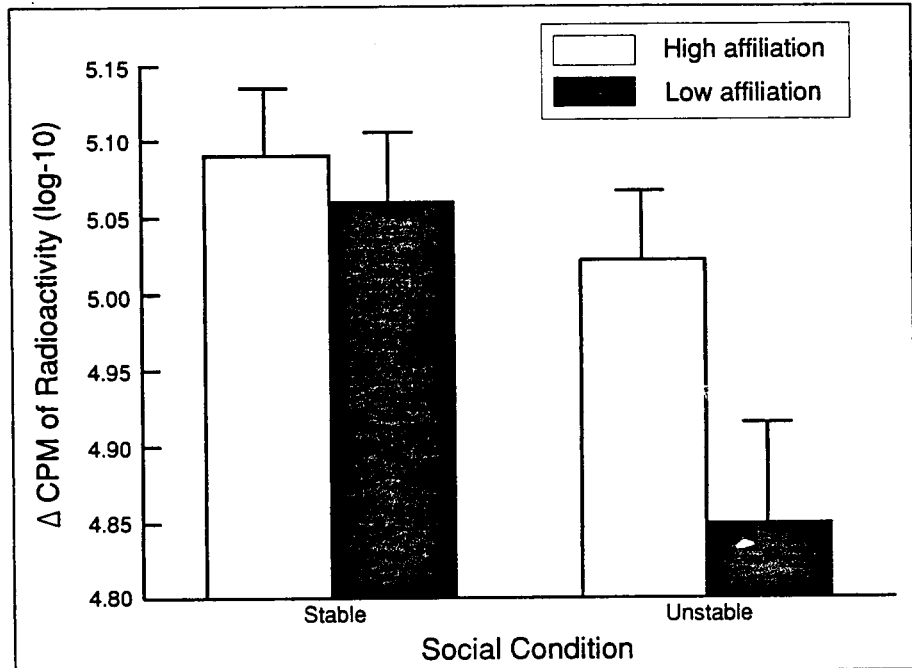


Fig. 1. Mean ConA-stimulated T-lymphocyte mitogenesis presented by social stability and affiliation. The data are collapsed across three weekly measures and mitogen concentrations. Depicted values are the base-10 logarithms of the difference in radioactivity between ConA-stimulated and unstimulated (baseline) samples expressed in counts per minute (CPM). Standard errors are indicated.

social situation (e.g., coping with social instability) or a stable response that occurred independently of the social situation, we analyzed the distribution of low- and high-affiliative animals across the experimental social conditions. Sixty-seven percent (14 of 21) of animals in the unstable condition were high in affiliation, while only 36% (8 of 22) of animals in the stable condition were high in affiliation ($\chi^2 = 3.95$, $p < .05$). Hence, affiliative behavior is more likely to occur in unstable conditions and thus may represent a situation-specific strategy for coping with social stress.

DISCUSSION

These data provide evidence for a suppression of T-cell function among animals exposed to a chronic-repetitive social stressor. Although there were mean differences between social stability groups on proliferation as stimulated by both mitogens, only the ConA data were statistically reliable. The data are consis-

tent with results of correlational and quasi-experimental human studies but extend this work by (a) providing experimental evidence from an ecologically valid nonhuman primate paradigm, (b) employing a prolonged (26 months) and clearly defined period of stressor exposure, (c) demonstrating stability of the effect over a 3-week poststress period, and (d) demonstrating the relative consistency of the effect across a range of mitogen concentrations.

T-cells from affiliative animals also demonstrated greater proliferation in response to ConA stimulation than did cells from nonaffiliative animals. Although the stability-by-affiliation interaction did not reach statistical reliability, it is clear from Figure 1 that the suppression of lymphocyte function among animals in the unstable condition occurred primarily for those who were low in affiliation. Hence, the data suggest that affiliation may operate as a stress buffer, protecting animals from the immunosuppressive effects of the chronic social stressor (Cohen, 1988). Future studies with more statistical power could help

clarify the reliability of the stability-by-affiliation interaction. Data indicating that there is more affiliative behavior among unstable than stable animals also suggest that affiliative behavior is a response to the social environment (possibly a coping response) rather than a dispositional characteristic that is stable across social situations.

Why do the psychosocial effects occur primarily in the case of ConA- but not PHA-stimulated proliferation? These differences may reflect differences in cell populations stimulated by the two mitogens. Both are known to stimulate T-cell division, and PHA also has a slight effect on B-lymphocytes. However, the subpopulations of T-cells influenced by each mitogen are not well documented. There is evidence that, depending on mitogen concentration, ConA may stimulate T-helper (CD4) or T-suppressor/cytotoxic (CD8) cells, but there are few data in regard to subpopulations stimulated by PHA (e.g., Rich & Pierce, 1973).

Finally, the effects of psychosocial factors on immune function are known to vary across species (Rabin, Cohen, Ganguli, Lysle, & Cunnick, 1989). Hence, a generalization of this work to humans would be premature. However, macaques are Old World monkeys with morphologic, behavioral, and physiologic similarities to humans that suggest macaques are appropriate as a model for understanding psychosocial influences on human immune response.

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