Research Report

INDIVIDUAL DIFFERENCES IN CELLULAR IMMUNE RESPONSE TO STRESS

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Abstract—*Correlational studies suggest that psychological stress suppresses cellular immune function in some, but not all, individuals. Here, effects of acute mental stress on lymphocyte subpopulations and T-lymphocyte mitogenesis were examined experimentally in healthy young adults. CD8 (T-suppressor/cytotoxic) lymphocytes increased in number and T-cell response to stimulation by phytohemagglutinin was attenuated following exposure to a 20-min laboratory stressor, but only in persons who also showed heightened catecholamine and cardiovascular reactions to stress. Hence, individuals differ substantially in their immunologic responsivity to behavioral stimuli, and such differences parallel (and may be predicted by) interindividual variability in stress-induced sympathetic nervous system activation.*

Although psychological stress is known to elicit many acute physiologic reactions, such responses vary in magnitude among individuals. This interindividual variability is best documented in relation to the hemodynamic adjustments induced by stress (e.g., abrupt changes in heart rate and blood pressure), and to a somewhat lesser extent by similarly elicited catecholaminergic activity (e.g., stress-related elevations in the plasma concentrations of epinephrine and norepinephrine) (Krantz & Manuck, 1984; Manuck, Kasprowicz, Monroe, Larkin, & Kaplan, 1989). Moreover, these cardiovascular and neuroendocrine parameters tend to covary when assessed under the same stimulus conditions, suggesting that much of the variability of subjects' physiologic reactions to stress reflects individual differences in behaviorally evoked sympathetic nervous system activation (Akerstedt, Gillberg, Hjemdahl, Sigurdson, 1983; Dimsdale, Young, Moore, & Strauss, 1987; Eisenhofer, Lambie, & Johnson, 1985; Goldstein, Dionne, Sweet, Gracely, Brewer, Gregg, & Keiser, 1982; LeBlanc, Cote, Jobin, & Labrie, 1979; McCubbin, Richardson, Langer, Kizer, & Obrist, 1983).

There is evidence that psychological stress also modulates aspects of cellular immune function in human beings, an effect which is again marked by appreciable interindividual variability. A common measure of cellular immune function is the ability of a key population of immune cells, the T (thymus-derived) lymphocytes, to divide when incubated with a mitotic stimulant. T-lymphocyte mitogenesis is suppressed during naturally occurring stressors such as bereavement, marital discord, and medical school examinations, yet many individual subjects exhibit little or no immunologic reaction to such events (Bartrop, Lazarus, Luckhurst, Kiloh, & Penny, 1977; Glaser, Kiecolt-Glaser, Stout, Tarr, Speicher, & Holliday, 1985; Kiecolt-Glaser et al., 1987; Schleifer, Keller, Camerino, Thornton, & Stein, 1983).

Extent of mitotic division may reflect the interrelation between two different populations of lymphocytes—the T-helper lymphocytes (denoted CD4 cells) whose role is to facilitate immune response, and T-suppressor/cytotoxic lymphocytes (CD8 cells) which act, in part, to suppress immune response. In this report, we describe an experimental study of the effects of psychological stress on both circulating lymphocyte populations and the T-mitogenic responses of healthy young adults. Results indicate that CD8 lymphocytes increased in number and the T-cell response to mitogen stimulation was attenuated following exposure to an acute laboratory stressor. However, these immunologic responses were observed only among individuals who also showed heightened responsivity to stress on a composite index of concomitant cardiovascular and catecholamine reactions (i.e., denoting "high" sympathetic activation).

METHOD

Subjects were 25 male volunteers, recruited from the university population and ranging in age from 18 to 30 years. Twenty participants were exposed to a 20-min laboratory stressor following collection of resting (baseline) physiologic measurements. These subjects were subsequently partitioned into clearly differentiated groups of high and low sympathetic "reactors," based on the relative magnitude of their cardiovascular and catecholamine responses to the experimental stimulus. The stressor consisted of two distinctly frustrating cognitive tasks: a modified Stroop color–word interference test (Frankenhauser, Mellis, Rissler, Bjorksell, & Patkai, 1968; Olsson, Hjemdahl, & Rehnqvist, 1986) and mental arithmetic (consecutive one-to-three digit addition/subtraction problems). Presented by computer in alternating 5-min bouts, the tasks were performed under pressure of time and, for the Stroop test, against a distractor (random test responses) generated by computerized voice synthesis. To determine whether procedural factors other than subjects' exposure to the laboratory stressor evoked changes in the dependent measures, the remaining five subjects were not subjected to stress, but in all other respects were treated the same

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as the experimental condition. All subjects gave informed consent to participate in this investigation, which was approved by the Biomedical IRB of the University of Pittsburgh.

On arrival at the laboratory, subjects were escorted to a temperature-controlled recording chamber and seated in a reclined (but not supine) position. An occluding cuff of appropriate size was placed on the subject's left arm and attached to a Dinamap 8100 Vital Signs monitor, for automated, noninvasive measurement of blood pressure and heart rate. An 18-gauge, indwelling catheter was next inserted into an antecubital vein on the subject's right arm. Following venipuncture, subjects rested quietly for 28 min, at which time 10 ml of blood were collected to determine: baseline lymphocyte populations; T-mitogenic response to phytohemagglutinin (PHA); plasma catecholamines; and plasma cortisol (as an indicator of potential pituitary-adrenocortical activation). Stressed (but not control) subjects were then administered the 20-min laboratory challenge. Additional blood samples were obtained in the third and eighteenth minutes of stress for measurement of plasma catecholamines, and on termination of the stressor for reevaluation of immunologic parameters and cortisol. Blood pressure and heart rate were recorded in minutes 26 and 28 of the prestress (baseline) period and every 2 min thereafter (i.e., during stress). Finally, identical measurements were made at corresponding intervals among the unstressed controls.

Plasma epinephrine and norepinephrine concentrations were determined by high performance liquid chromatography with electrochemical detection[1]; cortisol concentration was evaluated by radioimmunoassay.[2] To assess nonspecific mitogen stimulation, a whole blood assay was conducted to establish a dose response curve at PHA concentrations of 0.5, 2.5, 5.0, 10.0 and 20.0 μg/ml.[3] Response was defined as the difference in counts per minute between stimulated and unstimulated samples, determined separately for each concentration; these values were subjected to logarithmic (base 10) transformation prior to statistical evaluation. Lymphocyte subset analysis was performed using monoclonal antibodies labeled with either fluorescein or phycoerythrine to quantify CD4 (T-helper), CD8 (T-suppressor/cytotoxic), and CD19 (B, or antibody producing) lymphocytes.[4] Absolute number of cells was calculated from a complete blood count. Lymphocyte subsets could not be computed for one subject due to clotting, and the T-mitogenic response of one subject could not be determined due to bacterial contamination.

RESULTS

Heart rate and blood pressure data were reduced by calculating mean values for both *baseline* and *experimental* (i.e., stress) periods; similarly, the two measures of epinephrine and norepinephrine concentration during the experimental period were averaged to yield a single value for each subject.[5] In the stressed condition, individual differences in stress-induced sympathoadrenal activation were next identified by computing a composite index of these subjects' cardiovascular and catecholamine reactions to the experimental tasks. Heart rate and blood pressure were included in the composite index due to their responsivity to sympathetic nervous system stimulation under stress and to the good reliability of measurement achieved by their frequent evaluation in this investigation. Experimental period measurements, adjusted for baseline covariation (i.e., residualized scores), were calculated for each parameter: heart rate, systolic and diastolic blood pressure, epinephrine and norepinephrine. Subjects falling above the median of at least three of the five ranked distributions of experimental period measurements were identified as "high sympathetic reactors" [n = 9; $\overline{X}$ above-median ranks = 4.11 (sem = 0.20)]; the remaining, less responsive subjects were designated "low reactors" [n = 11; $\overline{X}$ above-median ranks = 1.18 (sem = 0.36)].

To evaluate subjects' cardiovascular and catecholamine responses to stress, baseline and (unadjusted) experimental period measurements (see Table 1) were subjected to a 3 × 2 Group (high and low reactors, controls) × Period (baseline, experimental) repeated measures analysis of variance (ANOVA) for each dependent measure. (Note that for control subjects, "experimental period" denotes a time of measurement only, as these subjects were not exposed to the laboratory stressor.) The Group × Period interaction term was significant in each of these analyses: heart rate [$F(2,22) = 15.76$, $p < .0001$], systolic and diastolic blood pressure [$F(2,22) = 26.53$, $p < .0001$; $F(2,22) = 4.26$, $p < .03$], and plasma epinephrine and norepinephrine [$F(2,22) = 5.20$, $p < .02$; $F(2,21) = 3.72$, $p < .05$]. Subsequent comparisons among group means [by Dunn's procedure (Kirk, 1968), $p < .05$] showed baseline measurements to be comparable in the three groups, with one exception: Control subjects had lower systolic blood pressure than either high or low reactors. As expected, comparisons of corresponding baseline and experimental period measurements showed no significant differences among control subjects, indicating that no spontaneous changes

1. Blood samples were anticoagulated with EDTA, chilled, and centrifuged; plasma was then removed and frozen at −80°C until analysis. HPLC determinations of epinephrine and norepinephrine, following extraction with alumina, were conducted using a Phase II, reverse phase, 3-micron column. Peak catechol heights were measured automatically by Chromatochart-PC (BAS/IMI).

2. A kit for radioimmunoassay was purchased from Diagnostic Products Corporation, Los Angeles, CA.

3. Blood was diluted 1:10 with RPMI-1640 tissue culture medium, supplemented with 10 mM hepes, 2 mM glutamine and 50 μg gentamicin per ml. One hundred μl of diluted blood were added to a 96-well, flat-bottomed culture plate (Coster #3596) containing 100 μl of mitogen solution added in quadruplicate (100 μl) of PHA prepared in RPMI-1640 to yield the five final PHA concentrations (0.5, 2.5, 5.0, 10.0 and 20.0 μg/ml). The plates were incubated for 120 hours at 37°C in air and 5% CO_2. Eighteen hours before the end of incubation, the wells were pulsed with 1 μCi tritiated thymidine and harvested for counting.

4. Lymphocyte subset analysis was conducted using a Becton-Dickinson FACScan instrument; monoclonal antibodies were purchased from Becton-Dickinson.

5. Calculation of means is justified by the high average correlation between mean values and individual readings (e.g., for experimental period measurements, heart rate: $r = .98$; systolic and diastolic blood pressure: .94 and .96; epinephrine and norepinephrine: .70 and .92).

Table 1. *Mean heart rate, blood pressure, and plasma catecholamines and cortisol during baseline and experimental periods among high- and low-reactive subjects and unstressed controls (standard deviations in parentheses)*

	Heart Rate bpm	Blood Pressure		Catecholamines		Cortisol μg/dl
		Systolic mmHg	Diastolic mmHg	Epinephrine pg/ml	Norepinephrine pg/ml	
High Reactors						
Baseline	59.2	116.1	62.9	24.4	234.7	9.3
	(5.3)	(5.4)	(7.4)	(6.7)	(118.5)	(2.7)
Experimental Period	77.2	131.9	71.2	48.3	268.3	9.9
	(9.5)	(6.5)	(6.8)	(28.0)	(112.4)	(4.1)
Low Reactors						
Baseline	52.3	114.6	57.7	18.1	244.3	9.2
	(7.4)	(6.4)	(10.0)	(7.7)	(105.4)	(3.0)
Experimental Period	59.2	119.8	61.6	25.5	237.8	9.4
	(7.9)	(4.9)	(9.4)	(9.5)	(87.4)	(3.8)
Control Subjects						
Baseline	52.9	104.0	59.0	18.4	285.3	10.9
	(9.5)	(3.2)	(4.9)	(14.6)	(115.8)	(6.6)
Experimental Period	54.6	106.4	59.3	17.4	290.4	10.4
	(6.6)	(3.5)	(3.3)	(8.5)	(100.8)	(6.6)

occurred over the course of testing (i.e., changes not attributable to stress). Among high sympathetic reactors, on the other hand, cardiovascular and catecholamine measurements during stress were significantly higher than preceding baseline values for all dependent measures. Low reactors showed significant baseline-to-stress elevations only in heart rate and systolic blood pressure, and in each case, these subjects' experimental period measurements were significantly lower than those of their high-reactive counterparts. Finally, parallel analysis of subjects' cortisol concentrations revealed no significant effects, indicating that cortisol did not differ between groups or as a function of exposure to stress.

The principal findings of this study concern the analysis of immunologic parameters. Accordingly, T-cell mitogenic responses were subjected to a repeated measures ANOVA, with one between-subject factor (*Group*) and two within-subjects factors: *Period* of measurement (again, baseline vs. experimental period), and within each period, *PHA concentration*. A significant main effect for PHA concentration [$F(4,84) = 219.53$, $p < .0001$] reflected expected differences in subjects' response to the graded concentration of mitogen.[6] Most importantly, T-cell proliferation in response to stimulation by PHA showed a significant Group × Period interaction [$F(2,21) = 3.59$, $p < .05$]. Comparisons among means ($p < .05$) revealed that T-cell mitogenesis decreased from baseline to stress among high sympathetic reactors, whereas low reactors and control subjects showed no change between the two periods of measurement. Baseline mitogenesis was also similar among the three groups. The absence of any interaction involving PHA concentrations indicates that these relationships generalized across all five concentrations; for purposes of illustration, mean mitogenic responses at 5 and 20 μg/ml are depicted in Figure 1, for high and low sympathetic reactors and control subjects.

Lymphocyte subpopulations were similarly analyzed by 3 × 2 (*Group* × *Period*) repeated measures ANOVAs (see Table 2). Here, the Group × Period interaction term was significant on analysis of CD8 (suppressor/cytotoxic) lymphocytes [$F(2,21) = 4.10$, $p < .05$]. Like the T-cell mitogenic response to PHA, comparisons among means ($p < .05$) revealed that the CD8 population changed (increased in number) from baseline to stress only among high sympathetic reactors. It is noteworthy that the three groups were comparable at baseline and that low reactors and control subjects exhibited no response to the experimental stressor. Analysis of other lymphocyte subsets revealed no significant effects.

DISCUSSION

These findings demonstrate that subjects differ substantially in their immunologic responsivity to stress, and that such differences parallel (and may be predicted by) interindividual variability on an index of concomitant sympathoadrenal activation. Whether the latter characteristic reflects an enduring and broadly expressed dimension of individual differences in sympathetic reactivity (Krantz & Manuck, 1984; Manuck et al., 1989) or a variability of response limited to the specific stressor presented in this investigation cannot be determined from the present data. In either case, these results suggest that influences of psychological stress on cellular immune function may be sympathetically mediated. This interpretation is consistent with evidence that lymphoid tissue receives sympathetic innervation (Felten & Olschowka, 1987) and that adrenergic receptors are expressed on lymphocytes (Feldman et al., 1983; Felten, Felten,

6. With respect to the concentration main effect, mean counts per minute ($\log_{10}$), collapsed over baseline and experimental period measurements, were 5.87 at .5 μg/ml of PHA, 5.97 at 2.5 μg/ml, 5.95 at 5.0 μg/ml, 5.68 at 10 μg/ml, and 5.24 at 20 μg/ml.

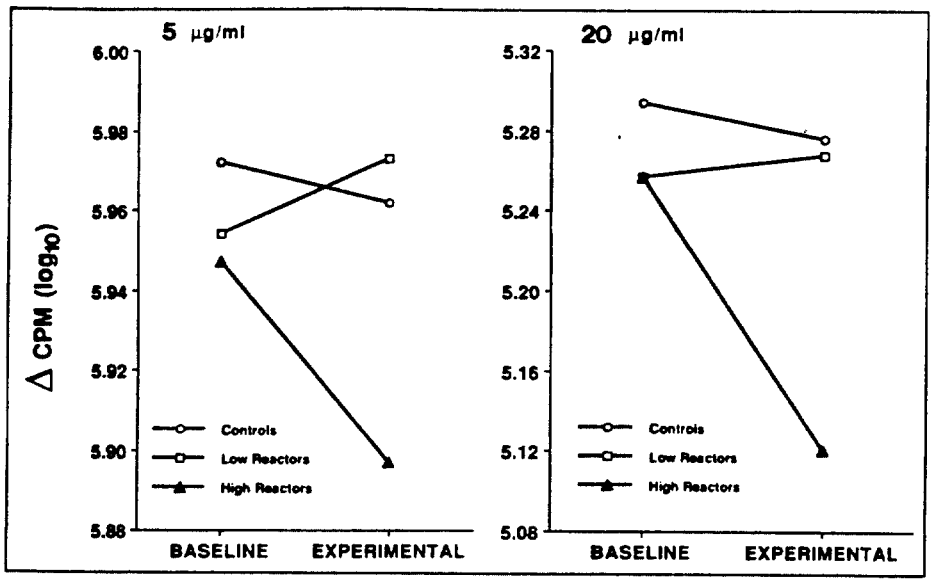

Fig. 1. PHA-stimulated T-lymphocyte mitogenesis, at baseline and following the experimental period, in high- and low-reactive, stressed subjects, and among unstressed controls. Depicted values are the differences in counts per minute (log_{10}) between stimulated and unstimulated samples at PHA concentrations of 5.0 and 20.0 μg/ml.

Carlson, Olschowka, & Livnat, 1985; Fuchs, Albright, & Albright, 1988; Khan, Sansoni, Silverman, Engleman, & Melmon, 1986). That immune alterations seen here occurred within 20 min of subjects' exposure to stress is also noteworthy, since direct infusion of epinephrine produces similarly rapid changes in lymphocyte distribution and mitogenesis (Crary, Borysenko, Sutherland, Kutz, Borysenko, & Benson, 1983; Crary et al., 1983). Although the mitogenic response to epinephrine infusion is known to dissipate (return to baseline) within 2 hours (Crary, Borysenko, Sutherland, Kutz, Borysenko, & Benson, 1983), it remains to be determined whether changes in cellular immune function induced by stress react similarly over time.

The fact that plasma cortisol concentrations were unaltered by stress also tends to exclude pituitary–adrenocortical activation as a probable mechanism underlying the present findings. It may be noted that cortisol reactions to behavioral stimuli tend to occur later than similarly elicited changes in plasma catecholamines; hence, measurements taken 30–40 min following subjects' exposure to the experimental stressor might have revealed a significant cortisol response. Also, it is possible that cortisol measurements in this study were somewhat elevated overall, owing to the initial stress of venipuncture. Nonetheless, the observation that cortisol concentrations neither differed between groups nor rose from baseline-to-task among high sympathetic reactors argues against an adrenocortical mediation of the T-cell distribution and mitogenic changes seen here under stress.

Finally, high sympathetic reactors showed a marked increase in circulating CD8 lymphocytes during stress, which was not observed among low reactors. Indeed, across all subjects, the change in number of CD8 cells between the baseline and experimental periods correlated inversely and appreciably with changes in PHA-stimulated lymphocyte mitosis ($r = -.66$ to $-.80$, $p < .005$ across all mitogen concentrations). This observation suggests that attenuated T-cell mitogenesis, when occurring as a function of stress, may reflect inhibitory influences of an expanded T-suppressor/cytotoxic cell population.

Table 2. *Mean T (CD3, CD4) and B (CD19) lymphocyte measurements during baseline and experimental periods, among high- and low-reactive stressed subjects and unstressed controls (standard deviations in parentheses)*

	Lymphocytes (cells/m³)		
	CD4	CD8	CD19
High Reactors			
Baseline	740 (229)	545 (82)	207 (94)
Experimental Period	715 (192)	684 (121)	199 (100)
Low Reactors			
Baseline	662 (185)	540 (237)	248 (103)
Experimental Period	625 (145)	541 (162)	226 (98)
Control Subjects			
Baseline	711 (149)	566 (130)	311 (167)
Experimental Period	707 (131)	596 (136)	285 (125)

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REFERENCES

Akerstedt, T., Gillberg, M., Hjemdahl, P., Sigurdson, K., Gustavsson, I., Daleskog, M., & Pollare, T. (1983). Comparison of urinary and plasma catecholamine responses to mental stress. *Acta Physiologica Scandinavica, 117,* 19–26.

Bartrop, R.W., Lazarus, L., Luckhurst, E., Kiloh, L.G., & Penny, R. (1977). Depressed lymphocyte function after bereavement. *Lancet, 1,* 834–836.

Crary, B., Borysenko, M., Sutherland, D.C., Kutz, I., Borysenko, J.Z., & Benson, H. (1983). Decrease in mitogen responsiveness of mononu-

clear cells from peripheral blood after epinephrine administration in humans. *Journal of Immunology, 130,* 694–697.

Crary, B., Hauser, S.L., Borysenko, M., Kutz, I., Hoban, C., Ault, K.A., Weiner, H.L., & Benson, H. (1983). Epinephrine-induced changes in the distribution of lymphocyte subsets in peripheral blood of humans. *Journal of Immunology, 131,* 1178–1181.

Dimsdale, J.E., Young, D., Moore, R., & Strauss, W. (1987). Do plasma norepinephrine levels reflect behavioral stress? *Psychosomatic Medicine, 49,* 375–382.

Eisenhofer, G., Lambie, D.G., & Johnson, R.H. (1985). B-adrenoceptor responsiveness and plasma catecholamines as determinants of cardiovascular reactivity to mental stress. *Clinical Science, 69,* 483–492.

Feldman, R.D., Limbird, L.E., Nadeau, J., Fitzgerald, G.A., Robertson, D., & Wood, A.J. (1983). Dynamic regulation of leukocyte beta adrenergic receptor-agonist interactions by physiological changes in circulating catecholamines. *Journal of Clinical Investigation, 72,* 164–170.

Felten, D.L., Felten, S.Y., Carlson, S.L., Olschowka, J.A., & Livnat, S. (1985). Noradrenergic and peptidergic innervation of lymphoid tissue. *Journal of Immunology, 135,* 755–765.

Felten, S.Y., & Olschowka, J.A. (1987). Noradrenergic sympathetic innervation of the spleen: II. Tyrosine hydroxylase (TH)-positive nerve terminals form synaptic-like contacts on lymphocytes in the splenic white pulp. *Journal of Neuroscience Research, 18,* 37–48.

Frankenhauser, M., Mellis, J., Rissler, A., Bjorksell, C., & Patkai, P. (1968). Catecholamine excretion as related to cognitive and emotional reaction patterns. *Psychosomatic Medicine, 30,* 109–120.

Fuchs, B.A., Albright, J.W., & Albright, J.F. (1988). B-adrenergic receptors on murine lymphocytes: Density varies with cell maturity and lymphocyte subtype and is decreased after antigen administration. *Cellular Immunology, 114,* 231–245.

Glaser, R., Kiecolt-Glaser, J.K., Stout, J.C., Tarr, K.L., Speicher, C.E., & Holliday, J.E. (1985). Stress-related impairments in cellular immunity. *Psychiatric Research, 16,* 233–239.

Goldstein, D.S., Dionne, R., Sweet, J., Gracely, R., Brewer, H.B., Gregg, R., & Keiser, H.R. (1982). Circulatory, plasma catecholamine, cortisol, lipid, and psychological responses to a real-life stress (third molar extractions): Effects of diazepam sedation and of inclusion of epinephrine with the local anesthetic. *Psychosomatic Medicine, 44,* 259–272.

Khan, M.M., Sansoni, P., Silverman, E.D., Engleman, E.G., & Melmon, K.L. (1986). Beta-adrenergic receptors on human suppressor, helper, and cytolytic lymphocytes. *Biochemical Pharmacology, 35,* 1137–1142.

Kiecolt-Glaser, J.K., Fisher, L.D., Ogrocki, P., Stout, J.C., Speicher, C.E., & Glaser, R. (1987). Marital quality, marital disruption, and immune function. *Psychosomatic Medicine, 49,* 13–34.

Kirk, R.E. (1968). *Experimental design: Procedures for the behavioral sciences.* Belmont, CA: Wadsworth Publishing Company, Inc.

Krantz, D.S., & Manuck, S.B. (1984). Acute psychophysiologic reactivity and risk of cardiovascular disease: A review and methodologic critique. *Psychological Bulletin, 96,* 435–464.

LeBlanc, J., Cote, J., Jobin, M., & Labrie, A. (1979). Plasma catecholamines and cardiovascular responses to cold and mental activity. *Journal of Applied Physiology, 47,* 1207–1211.

Manuck, S.B., Kasprowicz, A.L., Monroe, S.M., Larkin, K.T., & Kaplan, J.R. (1989). Psychophysiologic reactivity as a dimension of individual differences. In N. Schneiderman, S.M. Weiss, & P.G. Kaufmann (Eds.), *Handbook of research methods in cardiovascular behavior medicine* (pp. 365–382). New York: Plenum Press.

McCubbin, J.A., Richardson, J.E., Langer, A.W., Kizer, J.S., & Obrist, P.A. (1983). Sympathetic neuronal function and left ventricular performance during behavioral stress in humans: The relationship between plasma catecholamines and systolic time intervals. *Psychophysiology, 20,* 102–110.

Olsson, G., Hjemdahl, P., & Rehnqvist, N. (1986). Cardiovascular reactivity to mental stress during gradual withdrawal of chronic postinfarction treatment with metoprolol. *European Heart Journal, 7,* 765–771.

Schleifer, S.J., Keller, S.E., Camerino, M., Thornton, J.C., & Stein, M. (1983). Suppression of lymphocyte stimulation following bereavement. *Journal of the American Medical Association, 250,* 374–377.

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