Chronic Life Stress Alters Sympathetic, Neuroendocrine, and Immune Responsivity to an Acute Psychological Stressor in Humans

JENNIFER L. PIKE, PhD, TOM L. SMITH, PhD, RICHARD L. HAUGER, MD, PERRY M. NICASSIO, PhD, THOMAS L. PATTERSON, PhD, JOHN McCLINTICK, BS, CAROLYN COSTLOW, BS, and MICHAEL R. IRWIN, MD

Objective: Life stress is hypothesized to alter the dynamic regulation of the autonomic, neuroendocrine, and immune systems. This study examined the effects of antecedent chronic life stress on psychological and physiological responsivity after acute challenge with a psychological stressor.

Method: Using a within-subject mixed design, male volunteers with \(N = 12\) and without chronic life stress \(N = 11\) were administered a 12-minute laboratory stressor (mental arithmetic) vs a video control.

Results: Acute psychological stress induced subjective distress, increases of circulating concentrations of epinephrine, norepinephrine, \(\beta\)-endorphin, adrenocorticotropic hormone (ACTH), and cortisol, and a selective redistribution of natural killer (NK) cells into the peripheral blood as compared with the video control condition. Although the two groups were almost identical at baseline in psychological, sympathetic, neuroendocrine, and immune domains, the chronic stress group showed greater subjective distress, higher peak levels of epinephrine, lower peak levels of \(\beta\)-endorphin and of NK cell lysis, and a more pronounced redistribution of NK cells in response to the acute psychological challenge than the controls. Furthermore, the acute stressor induced a protracted decline in NK lysis per NK cell in the chronic stress group but had no effect in the controls.

Conclusions: In summary, when persons who are undergoing chronic life stress are confronted with an acute psychological challenge, an exaggerated psychologic and peak sympathomedullary reactivity occurs that is associated with decrements in individual NK cell function and is protracted beyond termination of the stressor and sympathomedullary recovery.

Key words: stress, immune function, neuroendocrine function.

INTRODUCTION

Life stress is generally thought to be associated with increased sympathetic outflow (1–3), activation of the hypothalamic-pituitary-adrenocortical (HPA) axis (1), and a reduction of ex vivo cellular immune function such as natural killer (NK) cell activity (4), an immune parameter important in host resistance to viral infection (5). However, such changes in resting concentrations of catecholamines, HPA hormones, or NK activity are not universally found in persons undergoing chronic stress. For example, in contrast with robust activation of sympathetic outflow that occurs after an acute stressor (6, 7), basal sympathetic activity as measured by circulating levels of either epinephrine or norepinephrine show increases, decreases, or no change in association with such chronic stressors as conjugal bereavement (8) or Alzheimer spousal caregiving (9). Likewise, chronic stress may not only fail to increase adrenocortical activity as was reported in the classic work of Wolff et al. (10), but certain individuals may actually suppress below baseline (11), consistent with preclinical observations (12). Finally, recent data indicate that at least the chronic stress associated with caregiving for an ill spouse is not associated with a reduction of NK activity (9, 13), although a recent meta-analysis demonstrates that a decrement of NK activity is a reliable correlate of life stress (4).

Rather than measurement of basal activity, preclinical animal models have routinely examined the dynamic regulation of these physiological systems and evaluated the effects of chronic stress on autonomic, neuroendocrine, and immune responses to an acute stressor (14). For example, using paradigms involving the central administration of a stress neuropeptide such as corticotropin-releasing hormone (CRH) (15) or the imposition of a novel acute stressor (16), chronic stress has been found to induce a
sensitization of the HPA axis that is experimentally uncovered and not identifiable under resting circumstances. This approach involving an acute challenge has also been used in human studies to reveal that aged persons show an exaggerated cardiovascular and sympathetic reactivity to acute stress (17) and that depressed patients show a blunted release of adrenocorticotropic hormone (ACTH) response to CRH infusion (18). However, surprisingly few studies in humans have examined whether chronic life stress alters the regulation of the neuroendocrine and immune systems as reflected by differences in responsivity to acute challenge. Two studies have shown that prior life stress is related to increased cardiovascular reactivity (19, 20), whereas Brosschot et al. (21) recently found that subjective, self-report of life stress was associated with decreased reactivity of stress-induced changes in immune cell traffic. Others have found that heterogeneity in psychological and cardiovascular reactivity to acute stress is associated with alterations of peripheral blood lymphocyte subpopulations and cellular immune responses (6, 22, 23). Likewise in chronic Alzheimer caregiver stress, in vitro stimulation with interleukin-2 reveals a defect in the regulation of NK cells in the chronic stress group not apparent at rest (13).

The purpose of the present study is to examine whether antecedent chronic life stress, independent of a psychiatric disorder (4), alters psychological and physiological responses to an acute psychological stressor. Prior observations are extended by the objective evaluation of life stress (24, 25), use of a widely recognized stress task and a video control condition in a within-subjects mixed design (26), and simultaneous assessment of psychological and subjective stress responses along with characterization of sympathetic reactivity (ie, plasma levels of epinephrine and norepinephrine), neuroendocrine reactivity (ie, plasma β-endorphin, ACTH, cortisol, and prolactin), and NK immune cell traffic. In addition, NK activity and cytotoxicity per cell are evaluated to determine whether acute stress has competing effects on NK cell activation in contrast to the selective increase of circulating NK cells. Changes in NK cell activation are more strongly associated with viral clearance than a transient redistribution of NK cell numbers (5). Thus, we hypothesize that individuals undergoing chronic stress will have exaggerated psychological and physiological responses to an acute psychological stressor with greater levels of subjective distress and release of sympathetic catacholamines and NK cells into the peripheral circulation as compared with responses in control subjects. A reduction of NK cell activation, that may or may not be present at baseline in the chronic stress persons, will differentially emerge in life stress subjects after the stress task.

**METHODS**

**Subjects**

Male community volunteers (N = 52) were selected using a standardized recruitment procedure of the Mental Health Clinical Research Center (MH-CRC) that involved a search of the San Diego catchment area using advertisements in local newspapers and area community centers. Before informed consent for the present study, the volunteers underwent a rigorous psychiatric and medical evaluation, by MH-CRC psychiatric research fellow-physicians, that included psychiatric and medical histories, physical examination, screening laboratory examination (chemistry panel, complete blood cell count, thyroid function tests, and human immunodeficiency virus (HIV) test), and formal structured psychiatric diagnostic interview using the Schedule for the Clinical Interview (DSM-III-R) (27, 28). The interval between subject recruitment by the MH-CRC and entry into the present study protocol varied from 2 weeks to 6 months. Thus, all subjects were reevaluated within 1 week before study protocol with an interim medical interview and laboratory examination. Furthermore, to rule out the occurrence of an interval psychiatric illness, the Diagnostic Interview Schedule (29) and the Alcohols Research Center Interview (30) were administered. Of the 52 volunteers 28 men were excluded for various reasons related to medical status, medication use, abnormalities on laboratory tests and/or the presence of an DSM III-R Axis I disorder and/or Borderline or Antisocial Personality Disorder (28). Antisocial Personality Disorder individuals were excluded because of the high risk of substance abuse in these patients who may also minimize reported use of substances. Likewise, we excluded Borderline Personality persons because of their affective instability in response to psychological stress and their risk for substance abuse. None of our subjects had Narcissistic Personality Disorder, and thus we had no Cluster B disorder patients in the sample.

Eligible subjects (N = 24) were in good medical health; had neither histories of recent (<10 days) viral infections nor histories of diseases (eg, autoimmune disorders or cancer) that could affect neuroendocrine or immune function. Screening laboratory tests including complete blood cell count, chemistry panel, and liver and thyroid function were within the normal range. All subjects were HIV negative. Study subjects denied lifetime use of psychotropic medications and β-blockers, and refrained from prostaglandin inhibitors (ie, aspirin or nonsteroidal antiinflammatory drugs) and any other medications known to affect immune function, during the 7-day period before study enrollment. None of the 24 subjects had a lifetime history of a DSM III-R Axis I mental disorder and/or Borderline or Antisocial Personality Disorder.

To assess life events, a modified version of the Psychiatric Epidemiology Research Interview (PERI-M) was used with selected probes from the Life Events and Difficulties Schedule (LEDs) and the Brown and Harris Contextual Rating System (CRS) (24, 25). A multistep process was then used to rate the severity and chronicity of life stress. Briefly, chronic life stress was determined by: a) Obtaining objective characteristics of a given punctate event or ongoing difficulty (24), with the time period of each event and difficulty, as well as chronicity, being established by specific queries regarding associated calendar events and the use of corroborative data (ie, financial statements, personal data...
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books, etc.); b) Evaluating and scoring the objective short- and long-term threat of each event or difficulty using the Brown and Harris Contextual Rating Manual (25); c) Ranking of each subject’s chronic stress and immunity based on severity and chronicity of secondary events/difficulties. Types of stressors experienced by subjects ranged from complicated bereavement, caregiving for an ill relative, and executive job loss with complications (chronic group) to simple changes in finance (eg, 6% decrease or increase in annual pay, <2% decrease in mortgage), or temporary/fleeting conflicts with roommates (control group). The reliability of rankings by two independent raters (J.L.P., T.L.S.) was $\kappa = 0.98$, $p<0.001$ and both experimenters and subjects were blind to chronic stress rating. After ranking on the basis of life stress chronicity and severity, subjects were stratified into two groups by a median split of rank score: chronic stress ($N = 12$) and control ($N = 12$). One control subject failed to complete an experimental task session and was excluded from the study.

Procedures

Experimental Overview. To evaluate the effects of acute mental stress on psychological, neuroendocrine, and immune domains, all subjects ($N = 23$) participated in two separate experimental sessions. These sessions were scheduled within 2 weeks of the life stress interview and were conducted approximately 1 week apart. Each testing lasted from 7:30 to 10:00 AM and followed identical procedures except for the presence or absence of the acute stress task.

On the morning of an experimental session, subjects arrived at the lab at 7:30 AM and were given a light breakfast, followed by a structured inquiry about sleep, medication and substance use, exercise patterns, and the presence of any interim stressors. All subjects denied sleep disturbance and use of caffeine containing beverages, alcohol, and all medications during 24 hours before testing. Subjects completed a 21-gauge intravenous (IV) catheter was then placed in an antecubital vein for repeated blood sampling during which time subjects rested quietly in a chair. Subjects were prevented from viewing the venipuncture and blood sampling by the use of a soft screen attached to the arm.

Over the next 30 minutes after placement of the catheter, subjects viewed a ‘nature’ video and the first baseline blood sample was obtained at the end of this interval. Viewing the video continued for another 15 minutes (ie, 45 minutes post-IV placement), at which time an abbreviated six-item version of the Subjective Stress Rating Scale (SSRS) questionnaire was completed (26). The resting video was resumed for another 15 minutes and the second baseline blood sample was obtained 60 minutes after IV placement. After this second baseline blood sample, subjects either resumed watching the video (control condition) or were given instructions for the mental arithmetic task (MAT) followed by its administration.

Each experimental task lasted 12 minutes with the order of experimental sessions counterbalanced across subject pairs. Immediately after the task, another blood sample was drawn along with administration of the SSRS. During recovery, subjects again watched the video. Collection of the final blood sample was done 30 minutes after termination of the task. After completion of both sessions, subjects were queried for their understanding of the project and study hypotheses: no subject disagreed either the study hypotheses or purpose.

Mental Arithmetic Stress. The acute stressor involved administration of a standard mental arithmetic task (MAT) under adverse conditions (26). Mental arithmetic is ideally suited to study psychophysiological reactions to stress as it is easy to implement, raises few ethical concerns than do some other types of laboratory stressors, and is easily adaptable by the addition of noise and task distractors that mimic problem solving in the real world and adds to the ecological validity of such tasks. Further, the stress associated with this particular MAT protocol has been shown to specifically elicit differences in immune and neuroendocrine reactivity that are not apparent at baseline (26). Briefly, subjects were asked to perform serial subtractions of sevens (or threes if they were unable to produce correct answers using sevens after five or more trials) from a four-digit number (eg, 4554). The examiner urged subjects to give accurate answers in time with each beat of a metronome (set at 20 bpm), and administered standardized prompts (eg, ‘Please concentrate,’ ‘Please keep time with the metronome,’ etc.) on a variable interval (VI 60) schedule throughout the task. All prompts were scripted in advance and the same script was used for all subjects. Importantly, these prompts served as a direct manipulation of subjects’ self-efficacy. Previous studies have shown that receiving negative feedback about performance that is unrelated to actual cognitive abilities reduces perceived self-efficacy (31), increases subjective distress (31), and has been linked to changes of the neuroendocrine, sympathetic, and immune systems (26, 31).

Assessment of Acute Psychological Stress. The SSRS evaluated subjects’ psychological responses to MAT. This self-report questionnaire consists of six visual analog scale ratings anchored by mood-related adjective pairs (eg, arousal, evaluated by the pairs: lively-unmotivated, and awake-drowsy). The shortened version of the scale has a coefficient a ranging from .78 to .92 (32).

Blood Sample Collection. All blood samples were drawn through the heparin-lock IV catheter into a 10-cc syringe and immediately transferred into either a heparinized Vacutainer glass tube for NK cell assays (22°C) or into a polypropylene tube with ECTA (on ice) for measurement of neuroendocrine and catecholamine levels. NK assays were begun within 30 minutes of the final blood draw, whereas plasma aliquots for neuroendocrine assays were stored at −70°C until assay.

Assays

Epinephrine and Norepinephrine. Plasma concentrations of epinephrine and norepinephrine were measured using previously published methods (9). This radioenzymatic assay has a sensitivity of 25 pg/ml for epinephrine and 65 pg/ml for norepinephrine with intra- and interassay coefficients of variation of 6% and 15%, respectively.

ACTH, ß-Endorphin (ßE), Cortisol, and Prolactin. Plasma concentrations of each of these neuroendocrine hormones were measured using published methods (33). Intra- and interassay coefficients of variation were from 3% to 8% and from 7% to 10% for the various measures.

Natural Killer Cell Enumeration and Activity. Isolation of peripheral blood lymphocytes, assay of NK activity, and enumeration of NK cell subsets used methods previously published (34). To minimize the in vitro effects of monocytes on the ex vivo assay of NK activity, we removed monocytes before the cytotoxicity assay, consistent with previous published protocols involving the effects of acute stress on NK activity. (26) All NK activity

produces increases in neuroendocrine responsivity and a subsequent down regulation of natural immunity. Separate 2 (group: control subjects, chronic stress subjects) x 2 (condition: MAT, video control) x 3 (time) repeated-measures ANOVAs were conducted for each of the dependent variables to generate the error term used in each planned comparison. The fourth step consisted of a series of planned comparisons (a priori contrasts/simple comparisons) to determine whether groups differed from each other at posttask and at recovery. Planned comparisons are considered the most appropriate test for evaluating specific hypotheses and allow for the “extraction of information critical to the status of research questions responsible for initiating of an experiment” (37).

Finally, the lack of an order effect was confirmed for each dependent variable by a 2 (order of condition) x 2 (group) x 2 (condition) x 3 (time) repeated-measures ANOVA.

The degrees of freedom for the error term (used in the a priori planned comparison) will change depending on whether the comparison made is evaluating between- or within-subjects factors (37). Degrees of freedom also change where data are missing (eg, problems with extraction; technician error resulting in contaminated sample) was small and did not exceed 5% for any one variable.

RESULTS
Sample Characteristics
The two subject groups were similar in age, education, income, and marital status (Table 1). None of the health-related behaviors nor use of substances were different between the controls and chronic life stress groups. Finally, neither medical status nor laboratory examination differed between the two groups.

Experimental Manipulation
In the combined group of subjects, administration of MAT induced a significant increase in subjective distress as measured by subjects’ SSRS scores (F(1,21) = 21.53, p<.001). There were no differences between subjects’ pre- and posttask scores during either the video or control condition.

Psychological Distress
As hypothesized, the chronic stress group reported significantly greater distress in response to the experimental stressor compared with controls (F(1,21) = 6.9, p<.05) (see Figure 1). There were no significant group differences in SSRS scores at baseline, or at any time point in the video condition indicating that subjective distress at rest is not universally increased in persons undergoing chronic life stress (all Fs <1.0, NS).

Sympathetic Nervous System Activity
MAT induced a significant increase in circulating levels of epinephrine (F(1,38) = 23.66, p<.001) and of norepinephrine (F(1,42) = 5.00, p<.05) at posttask across the total sample (Figure 2). Levels of epinephrine and norepinephrine were not significantly different at baseline (F(1,38) = 1.54, NS, F(1,42) = 0.69, NS, respectively), or at any time point during the video condition (all Fs <1.0, NS) (video condition data not shown).

Consistent with our hypotheses, the chronic stress group showed a significantly (F(1,38) = 4.11, p<.05) greater increase of epinephrine at posttask as compared with controls. For norepinephrine, however, there were no group differences at posttask (F(1,42) = 2.0, NS). After recovery from MAT, catecholamine levels in the total sample returned to baseline and there were no group differences at recovery (all Fs <1.0, NS).

Neuroendocrine Activity
MAT induced a significant increase in circulating levels of β-endorphin (F(1,42) = 17.35, p<.001), ACTH (F(1,42) = 63.59, p<.001), and cortisol (F(1,42) = 25.4, p<.001) across the total sample.
TABLE 1. Demographic, Health Behavior, Substance Use, and Medical and Laboratory Characteristics of Chronic Life Stress and Control Men

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Control (N = 11), mean (SD)</th>
<th>Chronic Life Stress (N = 12), mean (SD)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>36.8 (7.1)</td>
<td>40.5 (15.7)</td>
<td>t = 1.3, p = 0.29</td>
</tr>
<tr>
<td>Education (yr)</td>
<td>16.7 (1.8)</td>
<td>15.7 (1.9)</td>
<td>t = 1.2, p = 0.24</td>
</tr>
<tr>
<td>Income ($/month)</td>
<td>3284 (1315)</td>
<td>2683 (1512)</td>
<td>t = 1.0, p = 0.32</td>
</tr>
<tr>
<td>Cohabitating (%)</td>
<td>92</td>
<td>91</td>
<td>t = 1.2, p = 0.23</td>
</tr>
<tr>
<td>Health Habits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleep (hr/night)</td>
<td>6.9 (0.8)</td>
<td>7.2 (0.7)</td>
<td>t = 0.8, p = 0.43</td>
</tr>
<tr>
<td>Exercise (hr/wk)</td>
<td>5.9 (4.0)</td>
<td>8.0 (5.1)</td>
<td>t = 1.1, p = 0.29</td>
</tr>
<tr>
<td>Substance Usea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine (servings/day)b</td>
<td>1.9 (1.7)</td>
<td>2.1 (1.6)</td>
<td>t = 0.3, p = 0.60</td>
</tr>
<tr>
<td>Alcohol (drinks/day)c</td>
<td>1.6 (1.2)</td>
<td>2.1 (1.6)</td>
<td>t = 0.8, p = 0.44</td>
</tr>
<tr>
<td>Medical Status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (lbs)</td>
<td>198.0 (39.9)</td>
<td>178.7 (24.5)</td>
<td>t = 1.4, p = 0.17</td>
</tr>
<tr>
<td>Physician visits (6 mo)</td>
<td>0.5 (0.5)</td>
<td>0.6 (0.5)</td>
<td>t = 0.4, p = 0.68</td>
</tr>
<tr>
<td>Laboratory Examination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>70.3 (4.1)</td>
<td>70.3 (4.1)</td>
<td>t = 0.1, p = 0.99</td>
</tr>
<tr>
<td>Leukocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (10⁹/ml)</td>
<td>6.1 (2.2)</td>
<td>6.0 (1.5)</td>
<td>t = 0.2, p = 0.84</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>61.0 (9.4)</td>
<td>59.0 (10.3)</td>
<td>t = 0.5, p = 0.65</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>29.1 (7.2)</td>
<td>28.8 (9.8)</td>
<td>t = 0.1, p = 0.94</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>7.8 (1.7)</td>
<td>7.9 (2.4)</td>
<td>t = 0.1, p = 0.93</td>
</tr>
</tbody>
</table>

a Although two subjects (one Control, one Chronic Stress subject) had a lifetime history of tobacco use, each reported cessation of use beginning several years before the study.
b Servings per day based on caffeine content of approximately 250 mg per serving.
c Drinks per day defined as the equivalent of 1 oz hard liquor, or 4 oz of wine, or 12 oz of beer (nonmalt liquor).

Levels of β-endorphin, ACTH, and cortisol were not significantly different at baseline (F(1,42) = 1.87, NS; F(1,42) = 2.49, NS; and F(1,42) = 0.46, NS, respectively) or at any time points during the video condition (all Fs < 1.7, NS, data not shown).

Group comparisons demonstrated that levels of β-endorphin at posttask were significantly (F(1,42) = 20.96, p<.001) lower in the chronic stress group vs controls. Thus, in contrast with the exaggerated peak epinephrine response, a blunted peak increase of β-endorphin occurred in the chronic stress individuals after MAT. There were no group differences for ACTH or cortisol at posttask, (all Fs < 1.0, NS).

Recovery levels of β-endorphin remained significantly lower (F(1,42) = 7.3, p<.03) in chronically stressed persons as compared with controls. In contrast, cortisol levels in the chronic stress group remained increased above baseline during recovery and did not return to baseline (prestress) levels (F(1,42) = 20.8, p<.001). Although there were no group differences in absolute cortisol levels at recovery, exploration of individual responses suggested that nearly 50% of the chronic stress group had increases of cortisol from posttask to recovery, whereas controls showed either no change or decreases of cortisol during this recovery interval.

Finally, MAT had no effect on circulating levels of prolactin (F(1,21)=0.49, NS) and there were no group differences in prolactin levels at any time point during either the MAT or video conditions (all Fs < 1.0, data not shown).

NK Activity and NK Cell Number

In this group of carefully screened subjects, we found no differences in basal levels of either NK activity or NK cell number at baseline or at any time point during the video condition (all Fs <1.86, NS). However, the use of an acute psychological stressor, MAT, produced both the expected increase in NK activity (F(1,42) = 54.7, p<.001) and in circulating number of NK cells (CD16, 56) (F(1,42) = 51.7, p<.001) across the total sample and revealed significant group differences in responsivity (Figure 4). Comparison of NK activity in the two groups revealed blunted peak levels of NK activity at posttask...
Mental Arithmetic Task

Fig. 1. SSRS scores in the chronic stress and control subjects in response to the MAT and video control tasks. MAT produced significant increase in subjective distress across groups (a). A priori comparisons demonstrated that the chronic stress group had significantly higher SSRS scores after administration of the acute stressor $F(1,22) = 6.9, p<.05$ compared with control subjects (b), whereas baseline and video control values were similar in the two groups (all Fs <1.0, NS).

in the chronic stress group as compared with controls $F(1,42) = 5.6, p<.03$. In contrast, NK numbers at posttask were higher in the chronic stress group as compared with values in the controls, although this difference did not reach statistical significance.

Return of NK activity and of NK cell number to pretask levels during the recovery period was delayed in the chronic stress persons. Although NK activity and NK cell number at recovery had returned to baseline in the controls, these values remained increased over respective baselines in the chronic stress group $F(1,42)=3.92, p = .05; F(1,42)=5.97, p<.05$, for activity and number, respectively).

NK Activity per NK Cell

Because changes in NK activity in peripheral blood can be due to either change in circulating numbers of NK cells or in the level of activation of each lymphocyte, we calculated NK cytotoxicity per cell (38) to evaluate whether acute stress altered individual cell activation in addition to its effects on peripheral blood NK activity and NK cell number.

In the combined sample of controls and chronic stress subjects, the effect of MAT on NK activity per NK cell was just short of statistical significance $F(1,30) = 2.79, p<.08$.

However, comparison of the two groups found that efficiency of cell lysis or cell activation was significantly lower in the chronic stress group as compared with the controls both at posttask $F(1,30) = 11.16, p<.001$ and recovery $F(1,30) = 6.68, p<.03$. Yet, baseline levels of NK activity per NK cell were not
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Fig. 3. The effects of MAT on circulating levels of β-endorphin, ACTH, and cortisol in controls and chronic stressed persons at baseline, immediately posttask, and at recovery. MAT induced a significant posttask increase in β-endorphin \( F(1,42) = 17.35, \ p < 0.001 \) (a), ACTH \( F(1,42) = 63.59, \ p < 0.001 \) (a), and cortisol \( F(1,42) = 25.4, \ p < 0.001 \) across groups (a). At recovery, cortisol remained increased as compared with baseline values \( F(1,42) = 20.8, \ p < 0.001 \) (a). A priori contrasts demonstrated that levels of β-endorphin at posttask \( F(1,42) = 20.96, \ p < 0.001 \) (b) and at recovery \( F(1,42) = 7.3, \ p < 0.03 \) (b) were significantly lower in the chronic stress group as compared with controls. For ACTH and cortisol, groups were not significantly different at any time point (all \( Fs < 1.0, \ NS \)).

Fig. 4. The effects of MAT on NK activity, NK cell number (CD16, 56) and NK activity per number of NK cells in controls and chronic stress persons. MAT induced a significant posttask increase in NK activity \( F(1,42) = 54.7, \ p < 0.001 \) (a) and in NK cell number \( F(1,36) = 51.7, \ p < 0.001 \) (a). A priori comparisons demonstrated that NK activity at posttask was significantly lower in chronic stress group as compared with controls \( F(1,42) = 5.6, \ p < 0.03 \) (b). However, at recovery NK activity remained increased above baseline in the chronic stress group \( F(1,42) = 3.9, \ p = 0.05 \) (a), but not in the controls. For NK cell number, posttask and recovery levels tended to be higher in the chronic stress group as compared with controls \( F(1,42) = 2.27, \ p < 0.1; F(1,36) = 2.22, \ p < 0.1 \), but these differences did not reach statistical significance. At recovery, NK cell number remained increased above baseline levels in the chronic stress group \( F(1,36) = 5.97, \ p < 0.05 \) but not in the controls (a). MAT tended to increase lytic activity per cell in the total sample \( F(1,30) = 2.79, \ p = 0.08 \) (a). However, a priori comparisons demonstrated that the change in cell function was carried by control subjects, as chronic stress subjects showed no differences in NK activity per cell at posttask compared with baseline levels \( F(1,30) = .00, \ NS \). Furthermore, NK activity per NK cell was significantly lower in the chronic stress group at posttask \( F(1,30) = 11.16, \ p < 0.001 \) (b) and at recovery \( F(1,30) = 6.66, \ p < 0.03 \) (b) but not at baseline \( F(1,30) = .42, \ NS \), as compared with controls.

Association Between Subjective Distress and Physiological Changes

Because the chronically stressed group and controls differed in both their psychological and physi-
ological response to acute stress, we performed additional analyses to explore whether subjective distress in response to MAT accounted for changes in epinephrine, β-endorphin, and NK cell responses. Change in SSRS scores from baseline to posttask was positively correlated with posttask levels of circulating epinephrine ($r = 0.35$, $p < .05$) in the total sample and in the chronic stress group ($r = 0.63$, $p < .02$) but not in the controls. In contrast, change in subjective distress was negatively correlated with posttask levels of β-endorphin in the total sample ($r = -0.44$, $p < .02$) and in the chronic stress group ($r = -0.58$, $p < .03$) but not in the controls. Subjective distress was not associated with either posttask levels of NK activity or circulating NK cells. However, NK activity per cell at recovery was negatively correlated with change in SSRS scores in the total sample ($r = -0.48$, $p < .02$) and in the chronic stress group ($r = -0.63$, $p < .03$) but not in the controls.

**Association Between Neuroendocrine Measures and NK Cell Responses.** Inasmuch as both epinephrine and β-endorphin have been found to mediate alterations in NK cell numbers and activity in vivo (38–41), we examined whether changes in these hormones were associated with alterations of NK cell numbers or activation. Change in epinephrine from baseline to posttask was correlated with posttask levels of NK cell numbers in the total sample ($r = .50$, $p < .02$) and in the chronic stress group ($r = .73$, $p < .01$) but not in the controls. Similar correlations between change in epinephrine and posttask NK activity were also found, although partial correlations demonstrated that this relationship was due solely to the selective redistribution of NK cells into the peripheral blood. Change in circulating levels of epinephrine was not associated with NK cytotoxicity per cell, suggesting that intrinsic differences in lymphocyte response to increased circulating catecholamines, not merely the magnitude of sympathetic outflow, might underlie the decrement of NK cell activation in the chronic stress group.

Although change in β-endorphin from baseline to posttask was correlated with NK activity at posttask in the chronic stress group ($r = .47$, $p = 0.06$), there was no relationship between β-endorphin and measures of NK cell numbers or NK cell activation within or across groups.

**DISCUSSION**

In the total sample, administration of an acute psychological stressor induced increases in subjective distress, sympathetic outflow as measured by plasma concentrations of epinephrine and norepinephrine, increased activity of the HPA axis with increases in β-endorphin, ACTH and cortisol, but no change in plasma levels of prolactin. Consistent with prior observations (38–40), levels of NK activity in peripheral blood increased due to a selective redistribution of NK cells immediately after the acute stress. The video control condition was not associated with changes in either psychological or physiological measures.

Chronically stressed persons did not differ from controls at baseline across psychological, sympathetic, neuroendocrine, nor immune domains. However, use of an acute stressor unmasked important differences in psychological and physiological responsivity. The chronic stress group showed greater levels of subjective distress, higher peak levels of epinephrine, and lower peak levels of β-endorphin after MAT. Release of NK cells into peripheral circulation was also more pronounced in chronically stressed persons. Yet, despite this pronounced redistribution of NK cells, peak increases of NK activity were actually _blunted_ in the chronic stress group. Thus, after acute psychological stress, NK cytotoxicity per NK cell differed between the chronic stress group and the controls.

Furthermore, recovery after acute psychological stress was delayed in the chronic stress group. For NK activity and NK cell numbers, increases that occurred immediately after MAT were sustained through the recovery interval in the chronic stress group whereas NK cytotoxicity and NK numbers returned to resting values in the controls. Similarly, the decline in NK cell activation that emerged in the chronic stress group after MAT persisted through the recovery interval. Although no data are available to further address the time course of recovery of NK cell activity in the chronic stress group, it seems that acute stress-induced alterations of immunoregulatory cell traffic and function are not universally transient and short-lived changes that fully recover after termination of the laboratory stressor (22, 26, 38). Rather, acute stress may have a more or less sustained effect on NK cell responses depending in part on subject characteristics such as the presence of chronic stress. The health implications of this moderate decline in NK activation are not known. However, protracted and severe reductions of NK activity are associated with increased susceptibility to viral infections and possibly cancer (5).

Individual differences in psychological and cardiovascular reactivity to acute stress have previously been related to antecedent chronic stress or recent life stress experiences (19, 20, 23). These prior data
are consistent with the present findings of exaggerated subjective distress and increased release of epinephrine in the chronic stress group. Because β2-adrenergic activation mediates the selective redistribution of NK cells and other lymphocyte subtypes into the peripheral circulation (39, 40), we hypothesized that acute MAT-induced increases of peripheral blood NK cells would be greater in the chronic stress group. Indeed, the correlation between stress-induced release of epinephrine and increases of NK cells in the chronic stress group, but not in the controls, confirms and extends the work of Herbert et al (22) as well as the separate findings of Kiecolt-Glaser et al (23) and Malarkey et al. (42) that the magnitude of sympathetic activation determines changes in immune cell traffic (23). In contrast, Brosschot et al. (21) found that life stress as measured by self-report of daily hassles was associated with blunted (rather than exaggerated) stressor-induced increases of NK cells, although in their study no measures of cardiovascular or sympathetic reactivity were obtained.

Although exaggerated peak levels of circulating epinephrine may have accounted for the reduction of NK cell activation in the chronic stress group, the difference of cytotoxicity per NK cell that emerged after acute stress may be because of an increased sensitivity of lymphocytes to the immunosuppressive effects of epinephrine in the chronic stress vs control group. Van Tits et al. (43) found that epinephrine infusion resulted in an increase in the expression β2-adrenergic receptors on circulating mononuclear cells, and Mills et al. (44) demonstrated that severe, chronic life stress due to Alzheimer caregiving was associated with changes in both adrenergic agonist and receptor physiology. Mills et al. (44) found that over 30% of the variance in β-receptor sensitivity was accounted for by the high stress rating. NK cells may particularly vulnerable to alterations of adrenergic receptor function as this lymphocyte subtype has the highest density of β-adrenergic receptors of any lymphocyte subpopulation (45).

However, other neuroendocrine mechanisms cannot be ruled out. In the current study, stress-induced release of β-endorphin was blunted in parallel with lower levels of NK activity and of NK cytotoxicity per NK cell in the chronic stress group as compared with controls. Although neither β-endorphin levels nor changes in this hormone correlated with lytic activity after MAT, the specific opiate antagonist naloxone has been found to selectively block in vivo exercise-induced increases in NK activity but to have no effect on increases of NK cell numbers (41).

To interpret whether individual differences in physiological responsivity to MAT are related solely to chronic stress status, other pertinent characteristics including substance use histories, health behavior and medical status, and availability of support were evaluated. We found no differences in these variables between the two groups. Thus, group differences in the sympathetic, neuroendocrine, and immune responsivity are likely related to the chronic stress categorization rather than other factors associated with altered immune function. Whether persons with who are aged or suffer health or psychiatric problems also evidence greater psychological and physiological dysregulation after acute stress requires further study. Finally, we conducted several tests to evaluate the hypotheses, which increases the potential for Type I error. However, a priori planned comparisons were used and the pattern of results was generally the same across dependent variables.

In summary, we investigated the effects of acute psychological stress on subjective distress, sympathetic outflow, neuroendocrine activation, and NK cell traffic and function in persons with and without chronic stress. Although no baseline differences were found, the presence of chronic stress altered reactivity to acute stress in both psychological and physiological domains. Together these findings indicate that exaggerated psychologic distress and sympathomedullary peak reactivity occur in persons with antecedent life stress. In addition, we confirm that greater reactivity is associated with greater increases in circulating numbers of NK cells after acute stress. Finally, simultaneous assessment of NK activity and NK cell numbers has provided evaluation of NK cytotoxicity per cell or NK activation. As compared with controls, when persons with prior chronic stress are confronted with an acute psychological stressor, a decrement of NK cell activation occurs that is protracted after termination of the stressor and sympathetic recovery. The challenge now is to identify whether a decline in NK cell activation after a brief psychological stressor is a precursor to the reliable reduction of NK activity found at rest in some severely stressed or depressed patients.

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