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Nocturnal catecholamines and immune function in insomniacs, depressed patients, and control subjects

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Abstract

Insomnia predicts cardiovascular and non-cardiovascular disease mortality. This study evaluated EEG sleep, nocturnal sympathetic activity, and daytime measures of immune function in subjects with primary insomnia ($n = 17$) and patients with current major depression ($n = 14$) as compared to controls ($n = 31$). Insomniacs showed disordered sleep continuity along with nocturnal increases of average levels of circulating norepinephrine and decreases of natural killer cell responses, whereas depressed patients showed declines of natural killer cell activity, but no differences of EEG sleep or nocturnal catecholamines as compared to controls. Impairments of sleep efficiency correlated with nocturnal elevations of norepinephrine in the insomniacs but not in the depressives or controls. These data indicate that insomnia is associated with nocturnal sympathetic arousal and declines of natural immunity, and further support the role of sleep in the regulation of sympathetic nervous and immune system functioning.

Keywords: Insomnia; Depression; Sleep; Immunity; Sympathetic nervous system; Catecholamines; Natural killer cell activity; Cytokines

1. Introduction

Insomnia shows a high prevalence with between 10 and 50% of the general population reporting difficulty sleeping, depending on the methods used to assess insomnia and the population studied (Johnson, 1999; Ohayon and Caulet, 1996). In addition, disordered sleep occurs in association with many psychiatric disorders (e.g., depression) as well as multiple medical conditions including cardiovascular, infectious, and inflammatory diseases (Benca et al., 1992; Bloom et al., 2002; Foley et al., 1995; Han et al., 2002).

Epidemiological data increasingly implicate insomnia as a predictor of cardiovascular and non-cardiovascular disease mortality, particularly in community elderly populations (Foley et al., 1995; Kripke et al., 2002; Mallon et al., 2000; Mallon et al., 2002; Pollak et al., 1990). In regards to cardiovascular disease, rates of sudden cardiac death, myocardial infarction, ischemic stroke are lowest during nocturnal sleep, then peak at the end of sleep or in the morning following awakening (Marler et al., 1989; Muller et al., 1987; Muller et al., 1989; Willich et al., 1987). Furthermore in sleep apnea patients who have disturbances of sleep continuity, daytime elevations of catecholamines are found in

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association with increases in the incidence of hypertension and cardiovascular disease (Dimsdale et al., 1995; Hla et al., 1994; Leung and Bradley, 2001; Tochikubo et al., 1996). Conversely, hypertensive patients fail to show a nocturnal drop in blood pressure, indicating sustained sympathetic arousal during the night (Pickering and Kario, 2001). For non-cardiovascular disease, increased rates of cancer deaths correlate with habitual sleeping pill usage (Kripke et al., 2002), and impairments of immune function are associated with severity of disordered sleep in women at risk for cervical cancer (Savard et al., 1999).

To understand the pathways that might contribute to the link between sleep and health, recent studies have focused on the role of sleep in the homeostatic regulation of the sympathetic nervous and immune systems, and used experimental sleep deprivation to probe these relationships (Dinges et al., 1995; Irwin et al., 1996; Irwin et al., 1999; Redwine et al., 2000; Rogers et al., 2001). During sleep, sympathetic tone and circulating levels of sympathetic catecholamines decline (Dodt et al., 1997; Irwin et al., 1999; Somers et al., 1993), whereas sleep deprivation leads to nocturnal increases of norepinephrine and epinephrine (Irwin et al., 1999). Acute sleep loss is also associated with alterations in the expression of cytokines and declines of cellular and natural killer (NK) cell responses (Irwin et al., 1994; Irwin et al., 1996; Redwine et al., 2000), an immune cell that is considered to be an important marker of immunological defense against tumors (Shakhar and Ben-Eliyahu, 1998; Trinchieri, 1989). Some studies have, however, failed to find effects of sleep deprivation on immunity or have found increases of immunity with prolongation of sleep loss for 72 h or more (Benca and Quintas, 1997; Dinges et al., 1994).

Translation of these experimental findings into a naturalistic setting is needed to determine the relationships between impaired sleep and abnormal autonomic and immune system functioning in clinical populations. Patients with primary insomnia or with depression show abnormalities of sleep continuity (Benca et al., 1992; Bonnet and Arand, 1998; Thase et al., 1997), but the relationships between disordered sleep and changes of nocturnal sympathetic catecholamines, an index of sympathetic neural activity (Wallin, 1988), are not well defined in either of these groups. Likewise, while some data suggest that severity of disordered sleep predicts declines of NK activity in depressed patients (Cover and Irwin, 1994; Irwin et al., 1992), it is not known whether immune abnormalities are found in persons with insomnia who show no evidence of depression. Thus, this study compared nocturnal sympathetic activity and immune measures in two populations, primary insomniacs and depressed patients. In both groups, it is hypothesized that impairments of objective EEG sleep will be coupled with nocturnal elevations of sympathetic catecholamines and decrements of NK cell activity and cellular immunity (i.e., stimulated IL-2 production).

2. Methods

A total of 62 men were included in the present study. All gave informed consent under University of California, San Diego (UCSD) Protocol No. 96095. For the present study, there were three groups: control subjects ($n = 31$), volunteers with primary insomnia ($n = 17$), and depressed subjects ($n = 14$).

Controls subjects were identified and recruited by the UCSD Mental Health Clinical Research Center (MHCRC) in response to community educational outreach efforts (i.e., lectures, meetings with community groups, etc.) or to advertisements placed in local newspapers or UCSD campus publications. Controls selected for entry into the study were age-matched (± 5 years) with either an insomniac or depressed subject. Depressed subjects were self-referred from the San Diego community to the UCSD MHCRC for evaluation of depressive symptoms and inclusion in non-intervention studies prior to entry into a treatment protocol. Insomniac subjects were volunteers who responded to advertisements that solicited participants who had complaints of insomnia.

Following telephone screening, eligible subjects were scheduled for an interview, gave informed consent, and underwent a comprehensive psychiatric and medical evaluation by a MHCRC psychiatric fellow-physician. This evaluation included a structured sleep disorders interview and a Structured Clinical Interview for DSM-III-R/DSM-IV (Association, 1994), medical history and review of systems, physical examination, and screening laboratory tests (chemistry panel, complete blood count, thyroid function tests, and HIV test) to confirm that subjects had no underlying medical condition that might result in insomnia or depression.

Psychiatric diagnoses were obtained in a consensus meeting of MHCRC psychiatrists, research fellows, and nursing staff. For the control subjects ($n = 31$), none had a lifetime history of a mental disorder by DSM-IV. Participants with insomnia ($n = 17$) were included if they met DSM-IV criteria for primary insomnia; sleep laboratory criteria were not used to determine eligibility. Depressed subjects ($n = 14$) fulfilled DSM-IV criteria for major depressive disorder, current. None of the insomniacs had a life-time history of an affective disorder, anxiety disorder, or other major psychiatric disorder, and none of the participants in any of the groups fulfilled criteria for alcohol or other substance abuse or dependence within the last 6 months, although 1 control, 12 depressed subjects, and 4 insomniac subjects fulfilled criteria for alcohol abuse (not dependence) in remission for greater than 6 months.

All subjects were in good medical health. No subject reported recent (<10 days) viral infections that could affect the immune measures. None had chronic medical conditions such as diabetes mellitus, cancer, or chronic obstructive pulmonary disease. One depressed subject and two insomniacs reported a history of hypertension that was currently treated with diuretic medication. Such treatment with diuretics is not known to alter EEG sleep or to influence nocturnal circulating levels of catecholamines; inspection of nocturnal catecholamines and of immunity in the three subjects with histories of hypertension revealed levels similar to those in respective depressed- or insomniac groups. None of the subjects were on other anti-hypertensive medication. At the time of evaluation, depressed subjects were not using antidepressants or other psychotropic medications that could affect sleep structure; only two depressed subjects reported a recent treatment history with one using a benzodiazepine medication 20 days prior to study, and another who had received antidepressant medication that was discontinued 21 days prior to entry. Similarly, eligible insomniac subjects were not using psychotropic medications; two insomniacs reported use of a benzodiazepine 7 and 14 days prior to study. Laboratory tests (chemistry panel, complete blood cell count, and thyroid tests) were all within normal limits. All subjects were negative for HIV antibodies.

For two weeks prior to EEG sleep assessment, sleep–wake activity was assessed by diary to confirm that all subjects followed a habitual sleep–wake schedule that was similar to the study protocol. Insomniacs were also required to report difficulty falling asleep or maintaining sleep for at least four nights during each of the two weeks.

2.1. Procedures

Laboratory sleep studies included two nights of polysomnography during which subjects adhered to their habitual sleep schedules with lights out between 22:00 and 24:00 h. During the first night of adaptation to the laboratory, recordings of pulse oximetry for oxygen desaturation were obtained to exclude subjects with sleep apnea (>10 apneas or hypopneas per hour of sleep). Tibial limb movements were also recorded and scored; seven of the insomnia subjects had >10 movements per hour of sleep. Comparison of the insomnia subjects with and without periodic limb movements on measures of EEG sleep, immunity, and nocturnal catecholamines did not reveal any significant differences, and the two sub-groups of insomniacs were combined in all further analyses.

During the second night, all night polysomnography was completed with placement of electrodes for EEG (C3 or C4), electrooculography, and submental electromyography recordings. EEG sleep records were visually scored according to the criteria of Rechtschaffen and Kales (1968) as previously described (Irwin et al., 2002).

An intravenous catheter was inserted into a forearm vein at 21:00 h and subjects rested in a supine horizontal position with the first blood sample obtained at 22.00 h after catecholamine levels had reached a stable, resting baseline. By means of long thin plastic tube, blood was sampled every 30 min starting at 22.00 h until 6.00 h without disturbing sleep. Samples were immediately placed on ice, centrifuged at 4 °C, and stored at −80 °C until assay for catecholamines. For the assessment of nocturnal catecholamines, the sample was comprised of controls ($n = 12$), insomniacs ($n = 13$), and depressives ($n = 14$). In the morning, blood was sampled for measures of NK activity, stimulated NK activity, IL-2 production, and lymphocyte enumeration.

2.2. Assays

2.2.1. Peripheral blood mononuclear cell preparation

For the immune assessments, blood samples were maintained at room temperature for no more than 2 h prior to assay. Peripheral blood mononuclear cells were isolated on Ficoll–Hypaque (Pharmacia, Piscataway, NJ), washed twice with PBS (Gibco Life Technologies, Grand Island, NY) and resuspended in the respective media described below.

2.2.2. NK activity

Assays of NK activity were begun immediately after separation of mononuclear cells. To remove adherent cells, mononuclear cells were incubated on plastic petri dishes and nonadherent, effector cells were obtained and resuspended to 2×10^6 cells/ml. A constant number (5×10^3) of ^{51}chromium (New England Nuclear, Boston, MA) K562 target cells were mixed with graded numbers of effector cells in triplicate across three effector:target (E:T) cell ratios (40:1, 20:1, and 10:l). Killed targets were then quantitated by measuring the amount of ^{51}chromium released into the supernatant using a gamma counter. Each assay involved control wells to evaluate spontaneous release and maximal release. Results of the NK cell assay across the three E:T ratios were expressed as lytic units as previously described (Irwin et al., 1990). Daily interassay variability in the assessment of NK activity was minimized by a number of quality control procedures described previously (Irwin and Miller, 2000). The intraassay coefficient of variation is <5%.

2.2.3. IL-2 stimulated natural killer cell activity

Peripheral blood mononuclear cells were isolated as described above and cultured with or without 50 U/ml of natural human IL-2 (Boehringer, Indianapolis, IN) for 18–24 h at 37 °C in a 5% CO_2 incubator (Irwin et al.,

1996). Cytotoxicity of the stimulated effector cells was measured with the Daudi cell target in a standard chromium release assay. Unstimulated effector cells do not generate measurable cytotoxicity against NK resistant Daudi cell targets.

2.2.4. Stimulated IL-2 production

As previously described (Irwin et al., 1996), peripheral blood mononuclear cells were resuspended at a final concentration 7×10^5 cells/ml in a 1:1 mixture of RPMI 1640 and Dulbecco's modified Eagle's (DME) media supplemented with 10% fetal calf serum (Hyclone, Logan, UT; inactivated 1 h in 56 °C water bath), 4 mM glutamine, 20 mM Hepes, 5×10^{-5} M 2-mercaptoethanol (Sigma, St. Louis, MO), and 50 μg/ml gentamycin along with an optimal dose of Concanavalin-A (Con-A, 10 μg/ml; Sigma, St Louis MO). In preliminary studies, the use of a 1:1 mixture of RPMI 1640 and DME was associated with greater cell culture viability in stimulated and unstimulated cultured as compared to the use of either media alone. After 72 h, supernatants were harvested and frozen immediately at −70 °C until assay of IL-2 by means of enzyme-linked immunosorbent assay methods (R&D Systems, Minneapolis, MN). All IL-2 samples from control, depressed, and insomniacs were assayed at the same time, in a single run with a single lot number of reagents and consumables employed by a single operator. The intraassay CV values for all variables were less than 5%.

2.2.5. Cell enumeration

Percentage of T lymphocytes (CD3) and NK cells (CD16,56) was assessed by flow cytometry as previously described (Irwin et al., 1996).

2.2.6. Catecholamine assay

Catecholamines were assayed by modification of the catechol-*O*-methyltransferase (COMT) based radioenzymatic assay (Ziegler and Lake, 1992). This technique uses a pre-assay concentration step which eliminates interfering substances and improves sensitivity 10-fold. The intraassay coefficients of variation for norepinephrine and epinephrine are 4 and 13%. The interassay coefficients of variation are 10 and 16%.

2.3. Statistical analyses

To test for differences between the three groups for dependent variables (i.e., age, depressive symptoms, alcohol consumption, NK activity, stimulated NK activity, IL-2 production, lymphocyte subsets, sleep EEG variables, and nocturnal catecholamine levels), analyses of variance (ANOVA) were used. Statistical evaluation of an overall time effect for the circulating levels of norepinephrine and epinephrine across the nocturnal period did not reveal any significant differences in serial measures of these catecholamines. Thus, average level of catecholamines was used in all of the analyses. For specific group comparisons, Bonferroni corrected multiple comparisons were used to maintain a family-wise α level of 0.05. Before the onset of this study, effect sizes were determined and used to estimate the number of subjects needed to detect group differences. There are large effects sizes for differences between insomniacs and controls (Bonnet and Arand, 1998) and between major depressives and controls (Thase et al., 1996) on measures of EEG sleep continuity but not sleep architecture. Thus, a weighted n of 20 yields power of .80 to detect significant ($p < .05$) differences for EEG sleep continuity measures. Similar power is found for NK activity. The relationships between EEG sleep, immunity, and catecholamines were tested using zero-order correlations on those variables that differed between groups; significance level was set at $p < .01$ for these analyses.

3. Results

Average age was similar among the three groups (Table 1) and the three groups did not differ in ethnicity; Euro-Americans comprised 77% of the controls, 77% of the insomniacs, and 86% of the depressives. Severity of depressive symptoms was significantly greater in the depressed patients as compared to controls and insomniacs. Alcohol consumption during the last three months was similar among the three groups. No subject fulfilled criteria for substance dependence and none reported use of marijuana, stimulants, or barbiturates in the last year.

Table 1
Age, depressive symptoms, and alcohol consumption histories in control, insomnia, and depression groups

	Control (A) Mean (*SD*)	Insomnia (B) Mean (*SD*)	Depression (C) Mean (*SD*)	ANOVA ($df = 2, 61$)	Group comparisons
Age (years)	44.4 (11.4)	49.8 (12.7)	41.2 (10.0)	$F = 2.2,\ p = .11$	NS
Depressive symptoms (21-item HDRS)	1.3 (1.6)	3.6 (2.4)	27.1 (6.3)	$F = 29.0,\ p < .001$	A, B < C
Alcohol consumption (last 3 months)					
Drinking days/month	12.0 (12.2)	14.8 (19.3)	12.8 (17.4)	$F = 0.2,\ p = .83$	NS
Days since last drink	15.0 (24.3)	22.5 (36.1)	26.7 (34.9)	$F = 0.8,\ p = .46$	NS

Insomniacs showed decreases of total sleep time, sleep efficiency, amounts of Stage 2 sleep and REM sleep as compared with controls (Table 2). Depressives showed decreases of Stage 2, but none of the other sleep variables differed between the depressed subjects and controls. Age was not correlated with any of the sleep variables, although the sample population was middle-aged with a narrow age range.

Across the nocturnal period, insomniacs showed elevated circulating norepinephrine (230 ± 96.1 pg/ml) as compared to depressed patients (156.7 ± 61.8 pg/ml) and controls (176.6 ± 59.7 pg/ml; $F(2, 38) = 3.3,\ p < .05$; Fig. 1). For epinephrine, there were no group differences.

Sleep efficiency was negatively correlated with norepinephrine in the total sample ($r = -.47,\ p < .01$) and in the insomniacs ($r = -.60,\ p < .05$; Fig. 2) but not in the controls ($r = .22,\ p = .50$) or depressives ($r = -.30$, $p = .30$). Other sleep variables that differed between insomniacs and controls (e.g., total sleep time, amounts of Stage 2 sleep, REM sleep) were not correlated with nocturnal catecholamines.

Both insomniacs and depressed subjects showed decreased NK activity, and a trend for lower stimulated NK activity, as compared with the controls (Table 3). IL-2 production, percentage of T lymphocytes (CD3), NK cells (CD16,56), and NK activity per NK cell were similar between the groups. None of the sleep variables that differed between primary insomniacs, depressives, and controls was associated with measures of NK activity or stimulated NK activity. Nocturnal levels of catecholamines were not associated with the immune measures.

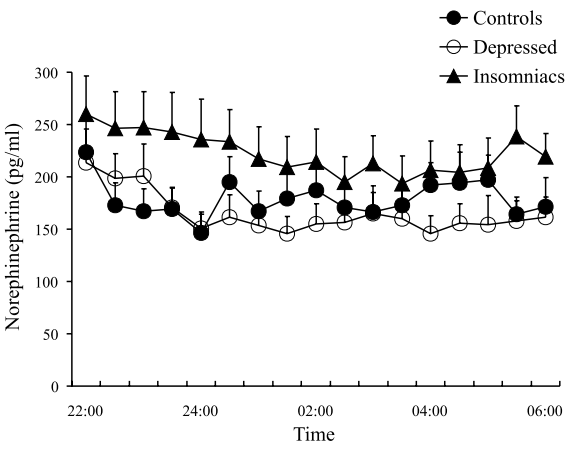

Fig. 1. Circulating levels of norepinpehrine during the nocturnal period in insomniacs, depressed patients, and controls. The bars represent the *SEM*.

4. Discussion

To our knowledge, this is the first study to demonstrate that subjects with primary insomnia show nocturnal elevations of circulating levels of norepinephrine and a reduction of NK activity. Furthermore, a difficulty with sleep maintenance as measured by sleep

Table 2
Sleep continuity, sleep architecture, and REM sleep in control, insomnia, and depression groups

	Control (A) Mean (*SD*)	Insomnia (B) Mean (*SD*)	Depression (C) Mean (*SD*)	ANOVA $df = 2,\ 61$	Group comparisons
Sleep continuity					
Total sleep time (min)	389.4 (44.8)	330.2 (71.0)	362.5 (48.9)	$F = 6.7,\ p < .001$	A > B
Sleep efficiency (%)	87.8 (7.6)	80.6 (13.6)	87.9 (4.2)	$F = 3.9,\ p < .05$	A, C > B
Sleep latency (min)	16.7(20.2)	25.7 (62.8)	15.3 (9.7)	$F = 0.4,\ p = .65$	NS
Sleep architecture					
Stage 1 (min)	30.1 (16.1)	33.0 (17.7)	23.3 (11.7)	$F = 1.6,\ p = .22$	NS
(%)	7.7 (4.2)	9.8 (4.8)	6.5 (3.5)	$F = 2.55,\ p = .09$	NS
Stage 2 (min)	242.5 (36.7)	193.8 (50.0)	214.9 (56.3)	$F = 6.6,\ p < .01$	A > B; A > C
(%)	62.3 (6.5)	59.6 (12.2)	59.1 (12.1)	$F = 0.7,\ p = .49$	NS
Stage 3 (min)	21.0 (16.9)	27.2 (18.8)	31.1 (20.1)	$F = 1.7,\ p = .19$	NS
(%)	5.4 (4.4)	8.0 (6.2)	8.6 (5.3)	$F = 2.5,\ p = .09$	NS
Stage 4 (min)	6.5 (12.3)	17.7 (26.7)	8.8 (10.8)	$F = 2.4,\ p = .07$	NS
(%)	1.6 (2.9)	4.9 (7.8)	2.4 (2.9)	$F = 2.7,\ p = .07$	NS
REM (min)	89.5 (22.7)	58.5 (23.7)	84.4 (37.1)	$F = 7.6,\ p < .01$	A, C > B
(%)	23.0 (5.4)	17.6 (5.9)	23.3 (9.8)	$F = 4.1,\ p = .02$	NS
REM measures					
Latency (corrected)	71.0 (37.1)	74.6 (41.9)	74.6 (52.4)	$F = 0.1,\ p = .94$	NS
Density	1.3 (0.6)	1.8 (0.6)	1.5 (0.7)	$F = 4.6,\ p < .05$	NS
Duration (min; 1st period)	20.7 (9.8)	20.4 (10.1)	21.5 (17.0)	$F = 0.1,\ p = .96$	NS

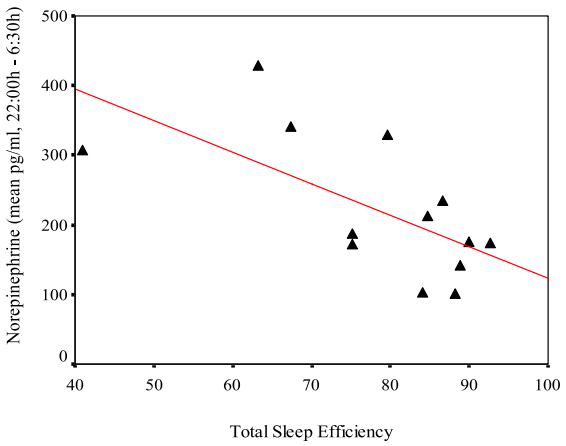

Fig. 2. Correlation between average nocturnal level of norepinephrine and sleep efficiency in the insomniacs.

efficiency was associated with increases of nocturnal norepinephrine in the insomniacs. Importantly, differences of nocturnal norepinephrine were not due to levels of physical activity as all subjects remained supine in the laboratory over the nocturnal period. Taken together with experimental evidence that partial night sleep deprivation increases nocturnal levels of norepinephrine and decreases NK cell responses (Irwin et al., 1994; Irwin et al., 1996, 1999), these data implicate sleep continuity in the regulation of the immune and sympathetic nervous systems.

Patients with primary insomnia had deficits of sleep continuity along with decreases of Stage 2 sleep and REM sleep. None of these differences of sleep are based on age or gender as the groups were comparable on these variables. Furthermore, none of the subjects showed co-morbidity for substance dependence or a medical disorder that might impact sleep. In contrast with meta-analytic findings (Benca et al., 1992), measures of EEG sleep continuity did not differ between depressives and controls. The lack of differences on measures of sleep architecture between the groups is in agreement with meta-analytic findings (Benca et al., 1992).

Insomnia is thought to be associated with chronic sympathetic hyperactivity as evidenced by elevated heart rate, body temperature, and whole body metabolic rate (Bonnet and Arand, 1995). During the sleep of insomniacs, increases of heart rate are maintained with decreased variability and the presence of low frequency spectral power characteristic of elevated sympathetic activity (Bonnet and Arand, 1998). In the current study, increases of nocturnal norepinephrine were found, further suggesting that insomnia and difficulty maintaining sleep elevates sympathetic tone.

Sleep maintenance problems and waking up feeling exhausted have been implicated as risk factors associated with a first myocardial infarction (Appels et al., 2000; van Diest and Appels, 1994). Whether elevations in nocturnal catecholamines that occur in association with disordered sleep contribute to the onset of cardiovascular events is not yet known, although sympathoadrenal activation produces a combination of transient hemodynamic, vasoconstrictive, and prothrombotic processes that are thought to increase the risk for plaque disruption and thrombosis, the final pathway of most myocardial infarctions (Andrews et al., 1996; Feng and Tofler, 1995). Nevertheless, in a 12-year follow-up of 1870 middle-aged men and women, sleep complaints (e.g., difficulty maintaining sleep) predicted coronary artery disease mortality (Mallon et al., 2002). Other recent data show that sleep duration less than 3.5–4.5 h or more than 8.5 h is associated with a 15% increased risk of mortality (Kripke et al., 2002), although cerebrovascular deaths were elevated only in those sleeping more than 8 h.

In animals, substantial evidence suggests that NK cell responses are an important marker of immunological defenses against tumors (Shakhar and Ben-Eliyahu, 1998). The present data show that insomnia is associated with declines of NK activity, suggesting a possible pathway in the link between insomnia and cancer morbidity (Savard et al., 1999) and mortality (Kripke et al.,

Table 3
Natural killer cell activity responses, interleukin-2 production, and lymphocyte enumeration in control, insomnia, and depression groups

	Control (A) Mean (*SD*)	Insomnia (B) Mean (*SD*)	Depression (C) Mean (*SD*)	ANOVA $df = 2, 61$	Group comparisons
NK activity (lytic units)	22.4 (20.2)	10.5 (6.0)	11.9 (10.7)	$F = 4.0,\ p < .02$	A > B, C
LAK activity (lytic units)	54.1 (41.4)	28.6 (15.8)	31.3 (14.2)	$F = 2.8,\ p = .07$	NS
IL-2 production (pg/ml)	14.7 (20.1)	3.2 (5.6)	7.8 (13.7)	$F = 1.6,\ p = .21$	NS
Lymphocyte subsets[a]					
CD3 (%)	65.3 (14.3)	62.6 (9.0)	73.3 (8.6)	$F = 2.4,\ p = .10$	NS
CD16,56 (%)	14.8 (4.3)	19.1 (5.5)	13.9 (7.7)	$F = 2.3,\ p = 0.11$	NS
NK activity/# CD16,56[a]	0.44 (0.4)	0.26 (0.1)	0.43 (0.5)	$F = 0.4,\ p = .66$	NS

[a] For these analyses, there were 11 controls, 11 insomnia, and 9 depressed subjects.

1979). Vitaliano et al. (1998) have also found declines of NK activity individuals with cancer histories which are exacerbated by the presence of sleep problems.

Given the cross-sectional design of the study, causal inference about the effects of insomnia on changes in sympathetic activity and NK cell responses can not be made. Second, the sample size is modest and there is risk of Type II error. Third, the blood sampling interval was restricted to the nocturnal interval whereas assessments of immune function were made during the day. Thus, inference about the contribution of nocturnal sympathetic tone to alterations of immunity cannot be made without simultaneous assessment of these measures. Fourth, it is not known whether findings are generalizable to women as the present sample included only male subjects. Fifth, the insomnia group was defined by complaints of difficulty sleeping without use of sleep laboratory criteria. Nevertheless, the insomniacs showed evidence of abnormalities in sleep continuity with sleep efficiency less than 85%. Sixth, the insomnia group included patients with primary insomnia as well as subjects whose sleep complaints may have been related to periodic limb movements. Finally, differences of IL-2 production between the groups were not found possibly due to the assay of only a single concentration of Con A rather than a dose–response profile.

Despite these limitations, the study hypotheses were advanced on the basis of previous work that experimental sleep deprivation can increase nocturnal sympathetic tone and alter natural and cellular immune responses. Extension of these findings to a clinical population furthers our understanding of the role of sleep in the homeostatic regulation of the autonomic nervous and immune systems. Patients with chronic insomnia show evidence of nocturnal sympathetic arousal that is coupled with decreases of NK cell responses.

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