Prolonged cardiac activation, stressful events and worry in daily life.

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Chapter 6: *Daytime Stress, Worry and Negative Emotional Traits and Cardiac Activation during Sleep*

ABSTRACT

***Objective**: Prolonged stress-related activity during nocturnal sleep might be a decisive factor in explaining the link between stress and disease, specifically cardiovascular (CV) disease. In a previous study, frequency and duration of stressful events and worry were found to be associated with increased average levels of heart rate (HR) and decreased average levels of heart rate variability (HRV) during one day and the subsequent sleep period; worry duration mediated the effects of stressors. We attempted to replicate these findings using among others, a longer time period, a more precise operationalization of worry and several additional potential explanatory variables.*

***Methods**: HR and HRV of 55 female and male teachers were recorded during neutral standardized laboratory tasks. Additionally, ambulatory HR and HRV recordings were performed for 4 days, during which the participants reported the number and duration of worry episodes and stressful events; this was done on an hourly basis using computerized diaries. Multilevel regression models were employed, accounting for the effects of biobehavioral variables. These variables included recovery from neutral laboratory stressors assessed in advance, job stress, and negative emotional traits (trait worry, anxiety, depression and hostility).*

***Results**: We found associations between frequent daytime stressors and elevated waking and sleep HR, but none for HRV. The effects on HR disappeared when adjusting for biobehavioral factors. Yet, daytime and sleep HR were associated with trait worry and a more incomplete HR recovery to standard neutral laboratory tasks. These findings were largely independent of effects of emotions, physical activity, posture and biobehavioral factors, such as gender, age, body mass or negative health behavior.Other psychological traits and job stress did not predict HR or HRV levels.*

***Conclusions**: Although the previous findings were not replicated in this sample, an independent effect on waking and sleeping HR was found for trait worry. The non-replication is likely due to less stressors and worry periods in the current sample and by the more stringent measure of worry. Alternatively, the effects of stressors and worry are less robust than assumed. It would be worthwhile in future studies to focus on a population with higher stress levels and extend worry measurements with tests of 'just problem solving'.*

During the past decades, the reactivity hypothesis has dominated stress-disease research stating that frequent elevated physiological responses during stressors lead to changes in physiological balance, triggering several pathogenic pathways. Recently however, it has been suggested that CV elevations during stressful events are probably not sufficiently long-lasting to cause chronic pathogenic states (1-4). Prolonged CV activity in absence of a stressor is proposed to be responsible for pathogenic states that can lead to cardiovascular disease. Accordingly, the level of CV activation in real life is not only influenced by simultaneously occurring psychological stressors, but also by more 'distal' stressors such as stressors in the past and anticipated future. Increased CV activation may be caused by slow recovery from preceding stressors or anticipatory responses to expected stressors. Indeed, several recent studies have shown that delayed cardiac recovery from cognitive (5-9) and physical (10-19) stressors is predictive of cardiac outcomes, such as hypertension, enhanced rest HR and BP, abdominal adiposity, and even overall mortality 3 to 15 years later ((5, 6, 8-11), reviewed in (20)). For practical reasons, laboratory stress studies have only tested restricted recovery periods, thereby limiting their ecological validity. Ambulatory studies (20) in natural environments have measured longer time periods. These types of studies have suggested that CV stress effects may last any period between 5 minutes and the rest of the day, including even the subsequent nocturnal sleep period (20). The present study tests whether frequency and duration of stressors and worry are associated not only with increased cardiac activity during the day but also whether they are related to prolonged activity during sleep at night. The possibility of deficient nocturnal recovery of physiological arousal due to a form of stress-related cognitive perseveration may be of potentially major significance for health. If stress-generated physiological arousal does not stop during sleep it leads to a situation not unlike being exposed to a virtually permanent stressor. Continuous physiological activation by stress without any natural restorative break might eventually cause serious health problems.

'Perseverative cognition' including phenomena such as worry or rumination, has been proposed as a mediator of prolonged effects of stress, by causing the continuation of stressful events in the form of cognitive representations of these events (4). These cognitive representations involve negative thoughts and action tendencies analogous to those elicited during an actual stressful event; they are shown to cause similar elevations in physiological arousal in several laboratory studies. Trait worry as well as real-life worry, experimental worry and rumination have been found to be associated with a range of physiological effects including higher heart rate (HR), lower heart rate variability (HRV), higher blood pressure (BP) and several effects on immunological and endocrinological parameters (see for a review (4)). Additionally, trait worry has been related to elevated risk of a second myocardial infarct (21). Moreover, worry and rumination are core elements of psychopathologies such as anxiety disorders and depression that are known to carry elevated CV disease risk (22, 23).

Recently, we have shown that cardiac effects of worry are not restricted to the laboratory but also occur in daily life. During worry episodes participants displayed increased HR and decreased HRV compared to neutral periods, and these effects were independent from those of stressors (24). We also showed (25) that stressors displayed prolonged effects up to one hour after their occurrence. Worry

showed a prolonged effect for up to two hours, which was independent of effects of stressors. All these effects were independent of those of biobehavioral factors. An earlier study by Brosschot, van Dijk and Thayer (26) showed that worry – especially worry duration - mediated the prolonged effects of stressors on daily as well as nocturnal HR and HRV. In addition, daily worry duration on itself had a substantial prolonged effect on nocturnal HR and HRV. It was suggested that sustained worry may 'leak' into nocturnal cognitive activity, which is accompanied by increased autonomic activity. Indeed, worrying has been observed to be most intense during the pre-sleep period; there is evidence for a peak in conscious worry in the first part of the night, just before sleep onset in healthy participants (27). Additionally, there is evidence that anticipatory worry before sleep onset can decrease HRV and REM sleep (28) and that stress and/or worries before sleep can negatively effect slow wave sleep (29, 30). These stressors or worries are perhaps continued on a less conscious level during subsequent sleep. However, this peak was missed in that study, in which stressors and worry episodes occurring after 10PM were not measured (26). Still, stress and worry occurring closer to sleep onset may have much stronger nocturnal cardiac effects, and may mediate the effects of earlier worry during the day and evening. The latter study (26) was limited in several other respects too. Firstly, stressor duration was not measured. Also, the study was confined to only one day and night per subject, while multiple days and nights might yield more reliable results. Furthermore, no distinction was made between work days and leisure days, and potential confounders such as physical activity were not measured. Finally, paper & pencil diaries were used, which carry the risk of filling in questions at a later time when retrospection leads to poorly reported variables (31). Therefore, we designed the present study to replicate the results of the above study (26) accounting for its limitations. The present study measured the frequency of stressors and worry episodes during two working days and two leisure days, including the period after 10PM and during the sleep period. It also measured the duration of stressors and worry episodes, as well as physical activity. Participants used a handheld computer which made it possible to exactly determine if and when they would fill out the questions. In addition, individuals differ to the extent to which they recover from any physical or psychological challenge, independent of its stressfulness, and this individual recovery slope may partly determine their recovery in daily life. For example there could be physical causes for slow recovery, due to an inherited or acquired diminished autonomic function associated for example with physical fitness, obesity, or age. To correct for these differences in the analyses of prolonged daily cardiac activity participants' recovery slopes after neutral stress were assessed in a laboratory session, using a standardized physical stressor (bicycle ergometer) and a neutral cognitive stressor (Stroop task).

We tested four hypotheses. *Firstly*, frequency and duration of worry and stressors during daytime and after 10PM are hypothesized to be associated with high HR and low HRV during both waking and subsequent nocturnal sleep. *Secondly*, it was hypothesized that at least part of the increased waking and sleeping cardiac levels of daily stress is mediated by worry during waking time and by late night worry (i.e. after 10PM). HR and HRV were measured because both chronic high HR and low HRV are risk factors for CVD as well as other organic diseases and overall mortality (32), and because they are easy to measure in daily life as well as during sleep without interfering with natural behavior.

Several negative emotional traits (depression, anxiety, worry, questionnaire-derived as well as interview-derived hostility (33-37)), and beliefs concerning work stress (i.e. job strain (38)) have been documented as CVD risk factors. The *third* hypothesis tested is that enhanced CVD risk of several negative traits or job strain (high demand/low control) is due to higher HR or lower HRV especially during sleep. There is some evidence that hostility is associated with enhanced systolic blood pressure during sleep, but no study has measured nocturnal HRV yet. Studies have been either scarce or inconsistent for the other traits and job strain (20). Only one study showed that job strain was related to lower nocturnal HRV (39). The present study also tested a *fourth* hypothesis which holds that these effects of traits are mediated by number and duration of stressors and worry episodes during the day, or by a more pronounced cardiac activity during these stressors or worry episodes. Age, gender, body mass index (BMI), bodily motion, time of day and the consumption of coffee and alcohol and smoking are known to effect HR and/or HVR (40-46); therefore, all analyses were corrected for effects of these biobehavioral factors. Since we measured participants repeatedly during four days and nights, measurements are more alike within participants than between participants. Due to the resulting hierarchical structure of the data we used multilevel regression models for the analyses. Because part of the data (HR and MSSD during waking hours) was used previously (24, 25) to analyze momentary daytime effects of worry and stress only, there is no overlap between the results of the current study and those of earlier ones.

Method

Participants

Subjects in this study were 55 teachers at 17 secondary schools in the Netherlands. The sample consisted of 39 men and 16 women aged 26 to 60 (mean=46.1; sd=8.5). Initially, 102 teachers were willing to participate; 29 dropped out before starting the experiment for various reasons (pregnancy, sick leave, allergy for electrodes not known before starting the experiment, antidepressant or hypertension medication) or were left out due to insufficient diary recordings. 18 Participants provided only valid daytime data and less than 3 hours of data during the night, and were therefore excluded for the analyses. 11 Participants were included in the analyses, although due to several reasons they produced valid data for only 48 of the 96 hours (six subjects withdrew from the project; one subject displayed an allergic reaction to the electrodes after 48 hours of measurements; one left for sudden sick leave; device malfunctioning caused drop-out of three participants). However, since they had more then three diary entries per day (the minimum required by us) they were included in the analyses. Eventually 55 participants were included in the study. All gave written informed consent and received a book token worth 20 Euros for their participation. The study was approved by the university ethics committee.

Procedure

After receiving consent of the management of the schools, teachers were recruited via regular mail. The responders were contacted by phone to schedule the laboratory session and the ambulatory measurements after which they received self-report questionnaires by regular mail.

Firstly, the teachers underwent a laboratory session, in which they signed the informed consent, were interviewed (IHAT, see below), and underwent a bike and Stroop task to estimate recovery after neutral stress (see below). Within two weeks afterwards, an experimenter fitted the ambulatory ECG device (47) in the morning before the teachers started their regular work activities and instructed them on the use of this device as well as a handheld computer that contained the hourly diary questions including questions about worry episodes and stressful events. Additionally, they were instructed to fill out a set of questions after waking up in the morning; these included questions about sleep quality and about worry episodes and stressful events after 10PM and during the sleep period.

They carried both devices for two periods of 48 hours. In between periods, devices were read out and provided with new batteries. At the end of the first 48-hour period the teachers left the devices at school where an experimenter could collect them. The day before the second 48-hour period, the equipment was handed over to the teachers, so that they could fit the equipment themselves after waking up in the morning.

State measurements

Diary format

A Palm^{tm} m100 handheld device (Palm Inc., Santa Clara, CA, USA) was used for both the hourly diary and the morning diary. We used customized software (Pendragon Forms, version 3.1.; Pendragon Software Corporation, Libertyville, Illinois) to implement questions and to transfer responses from the handheld to MS-Access data format. For the hourly diary, an hourly tone (plus or minus 15 min) was set from 8.00 AM to 10.00 PM on which participants were instructed to fill in the computerized questions. During work, a large part of these tones were programmed to occur in between lessons to reduce disturbance during teaching; the interval between two tones could therefore vary from 45 to 75 minutes. When the subjects answered the first question of each entry of the log, the present time was stored to enable comparison between their responses and the cardiac measurements. Additionally, subjects were instructed to fill in the questions of the nocturnal diary upon waking up in the morning. Thus, the measurements of stressors and worry covered the whole diurnal period.

Worry episodes and stressful events

The subjects received definitions of worry episodes and stressful events in print before starting the momentary measurements. The word for worry in Dutch is "piekeren". However, unlike the English word "worry" this word can also mean "thinking hard" or "pondering". To make sure that the subjects used the right concept we introduced the word "rumineren" (rumination) which is a seldomly used Dutch word. A "rumineer" episode or worry episode was defined as "*when you, for a certain period of time, feel worried or agitated about something. It is a summary-term for processes such as worry, ruminating, keeping on about something, fretting or grumbling about some problem or angry brooding etc. Thus, it is about a chain of negative thoughts that is hard to let go of.*". By using this definition we made sure that the subjects would also report other types of perseverative cognition apart from worry, such as angry brooding and rumination. Stressful events were defined as "*all*

minor and major events due to which you, to any extent, feel tense, irritated, angry, depressed, disappointed or otherwise negatively affected".

Since stressors and worry episodes occurring during the night take place closer to sleep and their nocturnal cardiac effects may be stronger than those occurring during the day, we distinguished between stressors and worry episodes occurring before 10PM and those occurring after 10PM and during the sleep period. To capture stressors and worry episodes occurring during daytime, the participants reported hourly on the handheld computer whether a worry episode or a stressful event or both had occurred during the preceding hour. If this was the case they additionally reported on the duration of the worry episode or the event (<5min, 5-15 min, 15-30 min, 30-45 min, 45-60 min, >60 min). Additionally, they retrospectively filled in the nocturnal diary upon waking up in the morning and reported whether a worry episode and/or a stressful event had occurred after 10PM the preceding night (yes, no) and if so, reported on the duration of that episode or event (<5min, 5-15 min, 15-30 min, 30-45 min, 45-60 min, >60 min).

Mood, activity, and other (bio)behavioral variables

During the last 15 minutes of each hourly measurement period and until 10PM, the subjects reported on the handheld computer to what extent they had felt the following four moods: being angry or irritated, sad or gloomy, tense or restless, and happy or cheerful (not at all, some, a bit, much, very much). Although these emotional states were not continuously measured we assumed that the aggregated 15 minutes samples yielded a valid representation of the occurrence of each of the emotional states during the day. The participants also reported on consumed units of tobacco, coffee and alcohol (0, 1-2, 2-4, more than 4) in the preceding hour, on having performed relatively strenuous activities in the preceding hour (not at all, some, a bit, much, very much) and on having slept or rested during the preceding hour (not at all, some, a bit, much, very much).

A more objective estimate of high activity was obtained with the ambulatory device, which includes an accelerometer sensitive to changes in vertical acceleration. This motility signal was used to distinguish periods with high activity from periods with low activity. High physical activities were identified as motility higher than the 48-hour average plus one standard deviation (indicating high physical activity) in combination with a visually detected simultaneous increase of HR, which was presumably due to this high activity. The percentage of 30-sec periods that were spent in high activity during daytime (for each day separately), is used as a covariate to control for cardiac differences due to intense movement. Note that for a previous report (24) (but not for (25)) we analyzed only periods in which participants displayed low activity.

Additionally, each morning upon waking up the subjects reported on how they evaluated their sleep quality in comparison to a normal night (much worse than normal, worse than normal, similar to normal, better than normal, much better than normal).

Cardiac activity

Ambulatory cardiac measurements were acquired continuously by the VU-AMS device (version 4.6. TD-FPP, Vrije Universiteit, Amsterdam, the Netherlands). This device has been used extensively and details of its characteristics have been

published elsewhere (48). In the present study the electrogram signal was recorded using disposable pre-gelled Ag-AgCL electrodes (ConMed, New York, USA) that were placed at the jugular notch of the sternum, 4 cm under the left nipple and at the lateral right side. Using this three electrode configuration the inter beat interval time series was available for analysis. The device detects the R-wave of the electrocardiogram and records the time in milliseconds (with one millisecond resolution). From the raw inter beat intervals the device derives and stores 30-second averages of HR (in beats/min) and root mean square of successive differences of inter beat intervals (in milliseconds: MSSD), which we used as an index of HRV. The MSSD has been shown to be a reliable index of cardiac parasympathetic influences (49), and is one of the time domain indices recommended by a task force report on HRV measurement (50). Additionally, the device includes an accelerometer sensitive to changes in vertical acceleration. This motility signal was used to distinguish periods with high activity from periods with low activity at daytime and to detect periods of high activity during the sleeping period, which might reflect waking up.

Individual recovery slopes to standard neutral stressors
To assess their 'natural' recovery in reaction to standardized non-stressful tasks participants performed a cognitive task and a physical task during a laboratory session. The cognitive task was a standardized Stroop task (51, 52) which was performed on a computer and consisted of four parts: firstly, participants had to read out loud and as quickly as possible the names of four colours printed in black. In the second part, they had to name as quickly as possible the colours of blocks that were printed in four different colours. Thirdly, subjects had to name as quickly as possible the four colours in which the words were printed while trying to ignore reading the words (of the same four colours). In part one to three, the participants had to name or read 70 items and the researcher timed their achievements with a stopwatch, while urging the subjects to perform faster. Finally, they had to sit quietly for 5 minutes and read neutral magazines in order to achieve recovery to baseline. The physical stress task consisted of exercising on a bicycle ergometer at the resistance of 40 watt (which is about 80 pedal steps per minute) for 5 minutes after which participants had to sit quietly for 5 minutes (recovery) reading magazines. Both the cognitive and physical task were performed in counterbalanced order after the IHAT interview (see below) and were preceded by a 5-minute (baseline) rest period. These tasks and the interview took place at the teacher's school in a room that was accommodated as a laboratory and that was inaccessible for others during the session.

Negative emotional dispositions and job strain
Trait hostility was measured by the Cook-Medley hostility scale (CM) (53). Nonverbal hostility was measured by the Interpersonal Hostility Assessment Technique (IHAT) (54), which is a rating system based on a structural interview for four subtypes of hostility: direct challenges to the interviewer, indirect challenges, hostile withholding of information or evasion of the question and irritation. In the present study two raters who had been trained by the developers of the test (54), independently assessed all interviews and achieved an intraclass correlation of .86. For the analyses these ratings were averaged across persons. The interview took place just

before the standardized stress tasks (see above). Symptoms of depression were measured by the Beck Depression Inventory (BDI) (55). Trait anxiety was assessed by the trait scale of the Spielberger State-Trait Anxiety Inventory (STAI) (56). Trait worry was measured by the Penn State Worry Questionnaire (PSWQ) (57) and the Worry Domain Questionnaire (WDQ) (58). The PSWQ was developed to measure the tendency for excessive, uncontrollable, pathological worry, while the WDQ quantifies worry across different areas of content. Job strain was measured by the Job Content Questionnaire, which measures job demand and job control in the workplace (59). All these scales are widely used, reliable and valid.

Data processing

Firstly, we eliminated all parts of cardiac data with outliers in standard deviation, mean, minimum and maximum values of HR, MSSD, IBI and motility. Secondly, we divided the cardiac data in waking period and sleeping period. Waking time was determined as the period between connecting the equipment (or elevating HR in combination with a visible increase of the AMS motility signal in the morning) until the last hourly entry of the day (around 10PM). The sleeping period was the period between 1 hour after going to bed (which was further validated by a visible decrease of the AMS motility signal which is continued during the rest of the night) to one hour before waking up (again validated by visible change to higher activity in the morning) and was confirmed with the subject's reports on sleep duration.

Next, cardiac data were divided into individual periods, by providing labels using the AMS graphical program (23). Cardiac data during waking time were labelled as neutral, worrying and/or stressful, based on the hourly diary data (see also (24, 25)). Additionally, in the resulting periods high activity or low activity periods were labelled. High physical activities were identified as motility higher than the 48-hour average plus one standard deviation (indicating high physical activity) in combination with a visually detected simultaneous increase of HR, which was presumably due to this high activity. The percentage of 30-sec periods that were spent in high activity is used as a covariate to control for cardiac differences due to intense movement. Additionally, periods of high activity during the sleeping period, which could reflect waking up and moving, were labelled and subsequently removed from the analyses. The AMS graphical program calculated mean HR and MSSD over the resulting labelled periods and these values were aggregated over the waking period and the sleeping period for the four 24-hour periods separately and were used as the dependent variables in the analyses.

Stressor and worry frequency during waking were measured by the number of times the presence of a stressor or worry was reported until 10PM. Stressor and worry duration were estimated for each worry episode by the subjects aided by a set of six time categories (<5min, 5-15 min, 15-30 min, 30-45 min, 45-60 min, >60 min). To facilitate analysis the begin point of these categories was used: 1 min, 5 min, 15 min, 30 min, 45 min, 60 min. The values were counted across entries. Subjects differed with respect to the number of entries that were actually completed (mean=11.25; standard deviation=2.99). Thus, the absolute values were not comparable between the subjects, which was why these variables were divided by the number of completed entries. A similar procedure was performed for reported units of tobacco, coffee and alcohol, as well as emotional states, and reports on

having performed relatively strenuous activities and the percentage of 30-sec periods spent in high activity.

Additionally, the subjects retrospectively filled in the nocturnal diary upon waking up in the morning and reported whether a worry episode and/or a stressful event had occurred after 10PM the preceding night (yes, no) and if so, reported on the duration of the worry episode or the stressful event (<5min, 5-15 min, 15-30 min, 30-45 min, 45-60 min, >60 min). Again, to facilitate analysis the begin point of these categories was used: 1 min, 5 min, 15 min, 30 min, 45 min, 60 min.

Cardiac recovery after neutral laboratory stressors was analyzed as follows. Each of the baseline and recovery periods during the laboratory stress session were divided into 5 separate 1-minute periods, of which the averages per period were calculated. For the baseline the 4th rest minute after the IHAT interview was taken, because due to circumstances for none of the participants the beginning and the end of this period were completely restful. Thereafter the area under the curve (AUC) minus the baseline was computed for each participant, for the cognitive and physical task and for HR and MSSD. The following equation was used derived from the trapezoidal rule (60): Excursion = [0.5 * fixed time interval ((cardiovascular measure at time 1) + (2 * cardiovascular measure at time 2) + (2 * cardiovascular measure at time 3) + . . .+ (cardiovascular measure at last time point)) - (baseline cardiovascular measure * the fixed time interval)]; where fixed time interval contained 1-minute averages for HR and MSSD, and each time point (e.g. time 1) represents a HR or MSSD value taken every 60 seconds, until the end of the 5-min recovery period. A lower value indicates more complete recovery. Because of high interdependence (r=.83 for HR and r=.74 for MSSD), we used the mean of the AUC estimations of the cognitive task and the physical task (for HR and MSSD separately) in the analyses below.

Statistical analysis

Multilevel regression models were applied to estimate the effects of frequency and duration of worry and stressors on HR and MSSD during waking and sleep. The choice of multilevel analysis arises from the hierarchical structure of the data: the waking and sleeping averages of HR and MSSD during maximally four 24-hour cycles are nested within subjects. We refer to these two levels as *day level* and *person level*, respectively. Predictor variables measured at these levels were entered into the model. Day level predictor variables used for analysis included stressor and worry frequency and duration during waking and sleeping, type of day (work vs. leisure), percentage high activity, reported level of activity, reported resting during awake period, sleep quality, emotional states and biobehavioral variables, including smoking and consumption of alcohol and coffee. Person level predictor variables entered into the model included gender, age, BMI, hostility (CM and IHAT), depression (BDI), anxiety (STAI), trait worry (PSWQ and WDQ), job strain and recovery after neutral laboratory stressors for HR and MSSD.

For all variables descriptive statistics were computed. The distribution for MSSD during waking and sleeping was non-normal, as well as frequency and duration of stressors and worry episodes during waking and sleeping, mean coffee intake and smoking, reported level of activity and sleep or rest during waking and mean angry or sad emotional states. Therefore, these variables were log transformed. All independent variables were centered around their grand mean.

A sequence of 4 models was tested separately for HR during waking, HR during sleep, MSSD during waking and MSSD during sleep. Additionally, to prevent errors due to collinearity we tested separate models for frequency and duration (of worry and stressors). Firstly, an intercept-only model was fit containing no predictor variables. This model decomposes the variance of the dependent variable into two independent components, pertaining to the day level and the person level, and was used as a baseline model. In the next model, we examined the effects of stressor and worry frequency (or duration) during waking (or during waking and during sleep). Next, the day variables (type of day, percentage high activity, reported level of activity, reported resting during awake period, sleep quality), biobehavioral variables (including smoking and consumption of alcohol and coffee) and the subject variables (age, gender, BMI and cardiac recovery after neutral laboratory stressors) were entered into the previous model to assess whether the effects of worry and stressors found in that model would still be present, and are thus not mediated by any of these latter variables (61). Next, we added the person level variables (trait worry, depression, hostility and anxiety and job strain) to test their main effects on waking and sleeping cardiac activity and if and to what extent the stressor and worry effects are due to these factors. In the final model, we added mean emotional states during waking, (i.e. being angry, sad, tense, and happy) to analyze whether the effects are mediated by mood.

To test the hypothesis that the prolonged effects of stressors – if any - were mediated by worrying, we additionally tested models without worry, and compared them with the models above including worry. Stronger prolonged effects of stressors that are also more significant without entering worry frequency or duration coulde indicate that worry mediates at least partly the effects of these variables (61). Similar tests were run for psychological traits and job stress.

The effects of the predictor variables in all models were considered fixed, since we did not have a specific interest in their random effects (apart from the variance components related to different levels). Multilevel regression models were fit using the program MLwiN, version 2.02 (62). The maximum likelihood method was used for model estimation. Significance of fixed effects of predictor variables was tested by dividing the estimated effect by its standard error. These effects were tested using one-tailed t-tests, since the hypotheses were explicitly directional. Model improvement was tested using likelihood-ratio tests (based on deviance values). An alpha level of .05 was used for all statistical tests.

Results

Descriptive statistics

Descriptive statistics of variables on the person and day level are given in Table 1. The mean scores of the questionnaires (PSWQ, WDQ, BDI, STAI, CM) and IHAT ratings were similar to other healthy samples (53, 55, 56, 59, 63-66).

During waking time, subjects reported a mean of .11 (sd=.13) stressful events per entry, which translates to a total of 1.5 per day if 14 entries (the maximum per day) are completed. These stressful events had a mean duration of 6.86 (sd=9.14) minutes. This duration is calculated only over entries in which stressful events were reported. On the contrary, for the calculation of the values reported in Table 1 entries in which stressful events were not reported, thus with a duration of 0 minutes were also taken into account. The subjects reported a mean of

.08 (sd=.12) worry episodes per entry, which translates to a total of 1.12 per day. The mean duration is 15.63 (sd=18.03) minutes. This duration is calculated only over entries in which worry episodes were reported. On the contrary, for the calculation of the values reported in Table 1 entries in which worry episodes were not reported, thus with a duration of 0 minutes were also taken into account. These frequencies are roughly comparable with findings from other studies, (e.g. 1.38 and 1.65 per day for stressful events (67, 68) and .96 per day for worry episodes (69)) but are lower than those reported in Brosschot et al (26) (stressor: .28 times per hourly entry; worry: .22 times per hourly entry). Additionally, after 10PM they reported a stressful event in 13.3% of the nights, with a mean duration of 6.86 (sd=9.14) minutes and worrying in 21.7% of the nights, with a mean duration of 15.63 (sd=18.03) minutes. As expected HR was higher during waking than during sleep (79.80 vs. 61.79, $t(149)=31.08$, $p<.001$) and MSSD showed the opposite effect (28.33 vs. 36.11, $t(149)=-6.98$, $p<.001$).

Table 2 shows the effects of biobehavioral variables and activity variables on HR and MSSD during waking and sleep. Women had higher sleep HR (64.32 vs. 60.95, $F(1,149)=5.87$, $p=.02$) than men. Older participants (>47.1 years) displayed lower MSSD during waking (25.45 vs. 31.41, $F(1,149)=10.07$, $p=.002$) and during sleep ($F(1,149)=14.92$, $p<.001$) than younger participants. Subjects with lower BMI (<24.3) displayed higher MSSD during waking (31.19 vs. 25.14, $F(1,144)=9.85$, $p=.002$) and during sleep (31.84 vs. 13.72, $F(1,144)=6.31$, $p=.01$) than subjects with a higher BMI. Participants who did not consume coffee during the day had lower waking HR (76.19 vs. 80.49, $F(1,146)=4.81$, $p=.03$) and sleep HR (58.39 vs. 62.44, $F(1,146)=6.20$, $p=.01$) than participants who consumed coffee. Subjects who reported smoking displayed higher daytime HR (84.19 vs. 79.01, $F(1.147)=6.36$, $p=.01$), lower daytime MSSD (21.60 vs. 29.55, $F(1,147)=8.63$, $p=.004$) and higher sleep HR (69.06 vs. 60.55, $F(1,147)=27.60$, $p<.000$). During work days, participants showed higher daytime HR than during leisure days (81.50 vs. 78.54, $F(1,142)=4.12$, $p=.04$).

Table 3 shows the correlations between worry and stressor frequency and duration, negative emotional dispositions, job strain and cardiac recovery after neutral laboratory stressors on the one hand and HR and MSSD during waking and sleep on the other hand. For these correlations (all two-tailed Pearson correlations) the data were aggregated on subject level. Correlations were significant only for the duration of stressful events, trait worry (PSWQ) scores, verbal hostility (IHAT) and cardiac recovery after neutral laboratory stressors. Increased duration of stressful events during the day was positively correlated to increased daytime HR ($r=32$, $p=.02$) and increased nocturnal HR ($r=.30$, $p=.03$). Higher trait worry scores (PSWQ) were related to elevated nocturnal HR ($r=.30$, $p=.02$). Increased verbal hostility (IHAT) scores were related to lower nocturnal MSSD ($r=-.30$, $p=.03$). Finally, a less complete HR recovery was related to both increased daytime ($r=.56$, $p=.00$) and nocturnal HR ($r=.36$, $p=.02$).

Effects on waking cardiac activity

Waking HR

Results of the intercept-only model (deviance 1006.43) for waking HR showed that the estimated value of the intraclass correlation for waking HR at person and at day

level was .66 and .34 respectively, which supports the use of a 2-level hierarchical data structure.

Stressor and worry frequency were added as predictors to the intercept-only model. There were no significant effects of stress and worry, except for a marginal trend of stressor frequency on HR (z=1.51, p=.07). The occurrence of more stressors tended to be associated with an elevated daytime HR of 8.50 (CI 2.85-14.15) beats/min.

However, when including the day variables (type of day, percentage high activity, reported level of activity, reported resting during awake period) cardiac recovery after neutral laboratory stressors and biobehavioral variables (age, gender, BMI, smoking and consumption of alcohol and coffee) in the next model the marginal trend of stressor frequency became non-significant. On closer inspection, we found this stressor effect to be due to the effects of type of day; work days were related to an increase of 4.17 bpm compared to leisure days (CI 3.15-5.18; z=4.10, p<.001). Apart from this, the following variables led to higher HR: more incomplete recovery after neutral stress in the laboratory (.09 bpm per incompletely recovered beat of HR and to a maximum increase of 12.86 bpm for the most incomplete recovery measured; CI .07-.12; z=3.68, p<.001), being female (4.48 bpm increase; CI 2.64-6.32; z=2.44, p=.007) and an increased activity level (18.52 bpm increase; CI 13.96-23.08; z=4.06, p<.001).

Including person level variables trait worry, depression, hostility, anxiety and job strain (Table 4, first column) showed that higher trait worry scores (PSWQ) were related to an increased daytime HR of between .26 bpm for the minimal PSWQ score and 13.26 bpm for the maximal PSWQ score (CI .14-.39; z=2.07, p=.02). No mediation test (hypothesis 4; see Introduction) was performed because there were no effects of worry and the marginal effects of stressors disappeared after adding type of day as reported above.

The same procedure, but now with stressor and worry duration as predictors resulted in largely similar findings as for frequency. When stressor and worry durations were added to the intercept-only model, only stressor duration was significantly associated with an increased daytime HR of 1.99 bpm (which corresponds to a maximal increase of 4.32 bpm, CI .92-3.07; z=1.86, p=.03). Again this effect disappeared when adding the variables mentioned above (Table 4, second column), and closer inspection showed again that this was due to the inclusion of type of day.

Waking MSSD

Similar procedures were followed for lnMSSD (Table 4, third column). Results of the intercept-only model (deviance 35.15) for waking MSSD showed that the estimated value of the intraclass correlation at person and at day level was .81 and .19 respectively, which supports the use of a 2-level hierarchical data structure. Adding stressor and worry frequency as predictors to the intercept-only model did not lead to significant results.

Table 4 (third column) shows the model including day variables (type of day, percentage high activity, reported level of activity, reported resting during awake period) cardiac recovery after neutral laboratory stressors and biobehavioral variables (age, gender, BMI, smoking and consumption of alcohol and coffee) as well as person level variables (trait worry, depression, hostility, anxiety and job

strain). Increasing age (z=2.00, p=.02) and reporting of more anxious symptoms (STAI; z=2.00, p=.02) were associated with decreases in daytime MSSD of -1.01 (antilog value; per year; implying a maximal decrease of 33.33 ms for 60 years old; CI. -2.02 to -.01) and -1.02 (antilog value; per STAI score; implying a maximal decrease of 34.68 ms for the maximal STAI score; CI. -2.03 to -.01) respectively. Addition of emotional states during the day revealed that anger showed a marginal trend (not in Table 4); participants who reported being angry or irritated more frequently, displayed marginally decreased daytime MSSD of –.50 ms (antilog value = 1.66; which corresponds to a maximal decrease in MSSD of -2.10 ms, CI -3.06 to -.26; z=1.49, p=.07**).**

The same procedure but now with stressor and worry duration (Table 4, fourth column) as predictors resulted largely in similar findings as for frequency. Effects of stressor and worry durations did not reach significance. Addition of the biobehavioral variables and trait values mentioned above led to similar results. However, this time one of the effects of angry emotional states was significant (not in Table 4). Participants who reported being angry or irritated more frequently, displayed decreased daytime MSSD of –1.84 (antilog value; which corresponds to a maximal decrease in MSSD of -2.33 ms, CI -3.25 to -.43; z=1.78, p=.04).

Effects on cardiac activity during sleep

Sleep HR

Results of the intercept-only model (deviance 928.35) for HR during sleep showed that the estimated value of the intraclass correlation for sleep HR at person and at day level was .76 and .24 respectively, supporting a 2-level approach. Adding stressor and worry frequency during the day, as well as after 10PM, did not lead to significant effects.

Adding the day variables (type of day, percentage high activity, reported level of activity, reported resting during awake period), cardiac recovery after neutral laboratory stressors and biobehavioral variables (age, gender, BMI, smoking and consumption of alcohol and coffee, as well as sleep quality) lead to the following effects: being female (increase of 5.14 bpm; CI 3.57-6.71; z=3.27, p<.001), increased alcohol intake during daytime (increase of 8.30 bpm; CI 4.72-11.87; z=2.32, p=.01), and slower laboratory recovery (increase from .06 bpm to a maximum increase of 8.52 bpm for the most incomplete recovery; CI .04-.08; z=2.95, p=.002) were related to increases in sleep HR. Adding person level variables trait worry, depression, hostility, anxiety and job strain to the previous model, only led to a significant effect of tendency to worry (PSWQ); increased tendency to worry was associated with increases in sleep HR of .18 bpm (per PSWQ score; implying a maximal decrease of 8.98 bpm for the maximal PSWQ score; CI. .08 to .28).

Finally, the addition of mean emotional states only displayed a significant effect for happy emotions during the day. Participants who reported being happy or cheerful more frequently during daytime displayed a lower sleep HR of 2.46 (which corresponds to a maximal decrease in HR of 6.94 bpm, CI 3.46-1.45; z=2.45, p=.007).

The same procedure but now with stressor and worry *duration* (during daytime and after 10PM) as predictors was performed. When stressor and worry durations were added to the intercept-only model, only stressor duration during

daytime was significantly associated with an increased sleep HR of 2.44 bpm (which corresponds to a maximal increase of 5.28 bpm, CI 1.59-3.28; z=2.88, p=.002). However, this effect disappeared when adding the day variables type of day, percentage high activity, reported level of activity, reported resting during awake period, cardiac recovery after neutral laboratory stressors and biobehavioral variables, including age, gender, BMI, smoking and consumption of alcohol and coffee, as well as sleep quality. On closer inspection, the disappearance of the effect was due to the inclusion of attenuated laboratory recovery (related to increase in HR after 10PM of .06 bpm; to a maximum increase of 8.52 bpm for the most incomplete recovery; CI .04-.08; z=2.73, p=.003). Similar to the findings for frequency (above) being female and increasing alcohol intake during daytime were related to increases in sleep HR of 4.83 (CI 3.21-6.45; z=2.98, p=.001) and 8.81 (CI 5.24-12.37; z=2.47, p=.007) bpm respectively. The inclusion of person level variables (trait worry, depression, hostility, anxiety and job strain) lead to non-significant effects (Table 5, second column).

Finally, the addition of mean emotional states displayed a significant effect for happy emotions only during the day. Participants who reported being happy or cheerful more frequently during daytime, displayed decreased sleep HR of 2.40 (which corresponds to a maximal decrease in HR of 6.81 bpm, CI 3.45-1.35; z=2.29, p=.01).

Sleep MSSD

Similar procedures were followed for lnMSSD. Results of the intercept-only model (deviance 83.12) for waking MSSD showed that the estimated value of the intraclass correlation at person and at day level was .81 and .19 respectively), indicating that the use of a 2-level hierarchical data structure is plausible. Adding stressor and worry frequency as predictors to the intercept-only model did not lead to significant results.

Next, the day variables type of day (percentage high activity, reported level of activity, reported resting during awake period, sleep quality) and biobehavioral variables (age, gender, BMI, smoking and consumption of alcohol and coffee) and cardiac recovery after neutral laboratory stressors were added to the model. Higher age (decrease of -1.02; antilog value, corresponding to a maximal decrease of 34.68 ms; CI -2.03 to -.01; z=2.43, p=.01), increased alcohol intake (decrease of -1.59; antilog value, corresponding to a maximal decrease of 1.06 ms; CI -2.91 to -.27; z=2.14, p=.02) and more reports of a worse quality of sleep (decrease of -1.08; antilog value, corresponding to a maximal decrease of 3.24 ms; CI 2.21 to .04; z=1.85, p=.03) ms respectively.

Next, person level variables (trait worry, depression, hostility, anxiety and job strain) were added to the previous model, but all lead to non-significant effects (Table 5, third column). Similarly, addition of mean emotional states lead to non-significant effects.

The procedure was repeated but now with stressor and worry *duration* (during daytime and after 10PM) as predictors and resulted in similar results. Effects of stressor and worry duration did not lead to significant results. Addition of the biobehavioral variables and trait values mentioned above lead to similar results: higher age (decrease of -1.02 ms, antilog value, corresponding to a maximal decrease of 34.68 ms; CI -2.03 to -.01; z=2.43, p=.01), increased alcohol intake

(decrease of -1.59 ms, antilog value, corresponding to a maximal decrease of 1.06 ms; CI -2.88 to -.04; z=2.30, p=.01) and more reports of a worse quality of sleep (decrease of -1.07 ms, antilog value, corresponding to a maximal decrease of 3.21 ms; CI -2.11 to -.03; z=1.70, p=.04) were related to decreases in MSSD. Additionally, more reports of sleeping or resting during daytime were related to decreases in MSSD of 1.58 (antilog value, corresponding to a maximal decrease of 2.37 ms; CI -2.88 to -.28; z=1.73, p=.04) ms.

Similar to the findings for frequency (see above) the addition of person level variables (trait worry, depression, hostility, anxiety and job strain) as well as the addition of mean emotional states lead to non-significant effects.

Psychological traits

Psychological traits, such as depression and hostility, are important CV disease predictors. The subject-level correlations reported in Table 3 between emotional dispositions and job strain on the one hand and HR and MSSD during waking and sleep on the other hand, indicate that nonverbal hostility (IHAT) is related with daytime MSSD and that tendency to worry (PSWQ) is related to HR after 10PM. However, these relations were not clearly found in the multilevel analyses reported above. It is possible that psychological traits effect HR or MSSD via the effects of biobehavioral variables; however, in the analyses above biobehavioral variables were entered in the model first and may have caused effects of psychological traits to become non-significant. Another reason might be that the above models contain many variables that lower degrees of freedom such that effects of psychological variables become non-significant. For these reasons and in light of the predictive power of these traits in the literature, we tested their effects on daytime CV activity and CV activity during sleep, by adding the predictors (trait worry, depression, hostility, anxiety and job strain) to the null-model. Elevated trait worry (PSWQ) predicted elevated daytime HR of .31 bpm (corresponding to a maximal increase of 15.56 bpm; CI .16 to .45; z=2.12, p=.02) and elevated HR during sleep of .30 bpm (corresponding to a maximal increase of 15.10 bpm; CI .18 to .42; z=2.45, p=.01); these results correspond to the effects found in the analyses above. Additionally, elevated nonverbal hostility (IHAT) was associated with decreased daytime MSSD of 1.78 ms (antilog value; corresponding to a maximal increase of 1.17 bpm; CI -3.17 to -.39; z=1.85, p=.03) and was marginally associated with decreased MSSD during sleep of 1.88 ms (antilog value; corresponding to a maximal increase of 1.24 ms,; CI -3.36 to .40; z=1.61, p=.05). On closer inspection, we found that the effects of nonverbal hostility diminished after adding (increasing) age (related to an increase of 1.02 ms antilog value, CI -2.02 to -.01; z=2.67, p=.004 for daytime MSSD and related to an increase of 1.02 ms antilog value, CI -2.03 to -.01; z=3.43, p=.0003 for MSSD after 10PM).

Unilevel exploratory analyses

In an earlier study we found that stressors and worry during daytime were related to higher HR and lower HRV during daytime and sleep (26). These associations were not replicated in the multilevel analyses above. Since effects of type of day were significant in the multilevel models predicting waking HR (Table 2, column 1 and 2), we expected that subjects reported stressful events and worry at a different frequency during work days and leisure days. Indeed, subjects reported more

stressful events (but not worries) during work days (mean 2.02, sd. 2.03 on work days vs. mean .92, sd. 1.34 on leisure days; $F(1,148)=14.92$, $p=.00$). We explored the possibility that effects of stressful events and worry were significant only on work days, but not leisure days. For this purpose, we added several variables including the interactions between stressful events or worry (during daytime or sleep) and type of day to the related models above including only stressor and worry (frequency or duration). None of these interaction terms reached significance in either of these models. To explore the possibility that effects of stressful events and worry were significant on only one of these days, we looked at bivariate correlations between stressful events, worry (frequency and duration, as well as during daytime and sleep separately) on the one hand and HR and HRV during daytime and sleep on the other hand but now separately for each day. Of all these (total=96) correlations, 9 reached significance, of which only 3 were as expected. Thus these results could be safely attributed to chance alone.

Discussion

This study was designed to examine the effects of worry and stressors on HR and HRV during daytime and the subsequent nocturnal sleep period in order to replicate findings of a previous study (26), in which stressors and worry were related to daily and nocturnal HR and HRV. Contrary to our expectations, we did not find stable associations between increased stressor and worry frequency or their respective durations on the one hand and increased HR or decreased HRV during daytime or sleep on the other hand. We found that stressor duration was related to increased daytime and sleep HR, and we also found a marginal association between stressor frequencies and elevated daytime HR. However, these associations disappeared when correcting for the effects of biobehavioral factors. More specifically, the effect of stressors on waking HR was associated with high HR related to a work day. The association between increased waking stress duration and sleep HR was related to a more incomplete recovery score from tasks in the laboratory.

Additionally, we found that a tendency to worry and nonverbal hostility were related to cardiac activity during daytime and nighttime. In a multilevel test of these associations only an association between trait worry and increased daytime and nighttime HR was yielded. The association between nonverbal hostility and HRV was related to the association between increased age and decreased HRV. Although expected, the association between trait worry and increased daytime and nighttime HR was not mediated by daily stress or worry. Additionally, reports of increased angry or irritated states were related to decreased waking MSSD, whereas reports of increased happy or cheerful states were related to decreased sleep HR. Finally, a more incomplete HR recovery to standard neutral laboratory tasks was associated with elevated HR during daytime and during sleep.

Our previous studies (24, 25), that were partially based on the same sample show that worry and stressors during daily life have simultaneous as well as prolonged (up to two hours) cardiac effects. The present study suggests that these effects do not necessarily generalize to mean cardiac levels during daytime or during sleep. The effects of increased stressor frequency and duration on daytime HR were associated with the effects of a work day on HR; these results are in accordance with our previous study (24), which found that stressful events related to work induced more HR elevations than stressful events related to other topics. In line with

the findings of this study, it is likely that a specific type of worry or stressor elicits prolonged cardiac effects that are pronounced enough to influence mean daytime or sleep levels. However, the infrequent reporting of stressful events did not allow enough cases for a thorough analysis of this hypothesis. It would be fruitful if future studies assessed the prolonged effects of specific stressors or worry episodes. Likely candidates would be worry about work or future issues and stressors related to work (24).

Yet, we were not able to replicate results of the previous study by our group (26), in which daily worry and stressors were found to be related to higher HR and lower HRV during waking and during sleep. Notably, not even late night or nocturnal worry episodes and stressors had significant cardiac effects on sleeping HR and HRV in the present study. We explored the possibility that separate days might display effects that fade away when examining four days together. However, unilevel explorations revealed no noteworthy results, meaning that there was no evidence for the possibility that effects were more pronounced during work days than during leisure days. These differences in findings were possibly due to the less frequent reports of stressful events and worry episodes in the current study. The frequencies and durations for stressful events (.11 events and .75 minutes per hourly entry) and worry episodes (.08 episodes and 1.13 minutes per hourly entry) that were reported in the present study were much lower than those reported in Brosschot et al (26) (stressor: .28 times and 1.20 minutes per hourly entry; worry: .22 times and 2.0 minutes per hourly entry). Possibly, the group of subjects in the present study did not report enough stressful events and worry episodes to influence mean cardiac levels. There are two possible factors that might explain this; the first one is related to the different samples measured and the second is related to a different definition of worry presented to the different subject samples.

Firstly, the study from Brosschot (26) differs from the present study with respect to the sample measured. Brosschot and colleagues measured individuals that were contacted through newspaper ads without obtaining any further individual or demographics data nor applying any exclusion criteria, which could have resulted in the recruitment of a more variable group. One advantage of a variable group is that there is more variance to account for possible effects than a more homogeneous group. The present study, recruited a uniform group of high school teachers, which is a highly educated subgroup with a medium social economic status (SES), and the results might not generalize to other groups with lower education and a different SES. Within this recruitment of teachers, it is possible that a selection bias influenced the results, for instance that mainly teachers responded who did not experience a lot of stress, or that those with the highest work load did not respond due to lack of time. It is also possible that due to their higher educational level their daytime worries were better processed and as a result led to less subconscious cognitive perseveration during sleep. Moreover, compared to the sample by Brosschot et al. (1) this group of teachers displayed a slightly lower trait anxiety (36.5 vs. 40.0 respectively) and trait worry (43.0 vs. 45.6 respectively) score. Considering the relation between these traits, state worry and stress, this might additionally explain why our sample reported stressors and worry less frequently.

Secondly, the definition of worry presented to the subjects in the previous ambulatory study (26) was slightly different from the one that was employed in the present study. Brosschot and colleagues used the Dutch word "piekeren", which has

an extra connotation of "thinking hard", while the present study focussed mainly on the meaning of worry as it is used in English, and thus more exclusively on the emotional negativity of the process. In order to do so, the present study not only used another definition but employed also a not well known Dutch synonym of the word worry, 'rumineren'. It is possible that this led the participants in the present study to only report those worries which go with clear negative emotions and thus overall reporting less 'worrisome' cognitive problem solving attempts, which on themselves might have had cardiac effects. Just "thinking hard" or cognitive problem solving may be an essential element of perseverative cognition (4). Worry has been defined as consisting of thwarted attempts to engage in mental problem solving (70), thus emphasizing the importance of the element of "thinking hard" or cognitive problem solving. Importantly, a recent laboratory study (71) performed by our group after the completion of the current study showed comparable HR and HRV effects during induced worry (about personal worry topics) and a neutral cognitive problem solving task (about self-irrelevant topics), and both were higher compared to a relaxation condition. Moreover, these effects were not explained by differences in negative mood. These results suggest that mere mental activity is responsible for a part of the physiological effects of worry, and not or not only the aspect of negative perseveration or emotionality per se. Therefore, it is possible that an important reason why the present study failed to replicate the findings of Brosschot et al. (1) is the current operationalization of worry. This may have caused a focus away from the element "thinking hard", which not only resulted in lower reporting of worry episodes but also in a lesser overall cardiac effect.

The present study shows that more frequent reports of angry or irritated states were associated with decreased daytime MSSD levels, while more frequent reports of happy or cheerful states were related to decreased HR levels during sleep. These relations were independent of effects of worry, stressful events, biobehavioral variables and trait scores. These results are in line with a finding by Steptoe and coworkers (73), who found that a more complete blood pressure recovery after laboratory mental stress tests was related to increased reports of positive affect during the day. This is also consistent with the results of Brosschot et al. (72), which indicate that negative emotions, but not positive emotions inflict prolonged activation 10 minutes later. Apparently, emotionality has effects independent of worry, that is, conscious perseverative cognition, with especially positive emotionality being beneficial for cardiac health.

It is noteworthy that several important psychological risk factors for CVD, namely hostility, depression, anxiety and job strain (33, 38, 73-77) were not associated with elevated cardiac levels during daytime or sleep. In contrast, the - far less often measured - tendency to worry (measured by the PSWQ) was associated with elevated daytime and sleep HR. The initially apparent effects of nonverbal hostility (IHAT) disappeared when statistically controlling for age, suggesting that elevated age may have been a confounding factor in earlier studies (40). In our recent review (25) we found nocturnal cardiac effects for these negative traits, but not consistently so, that is 2 out of 6 for hostility, 1 out of 3 for anxiety and 1 out of 2 for depression. To our knowledge, only one study has evaluated effects of worry on risk for CVD (34). Thus, our finding for trait worry suggests that prolonged cardiac activity during daytime and sleep mediates the prospective association with CVD as was found by Kubzansky et al. (34). These findings are relevant since

continuous physiological activation during sleep, which should be a natural restorative break, might eventually cause serious health problems. Regarding the limited studies on the CVD risk of worry, our findings stress the importance of including a measure of tendency to worry when studying risk for CVD. Since tendency to worry is a frequently observed aspect of anxiety and depression disorders, it is possible that measuring this factor might reveal an important part of the mechanisms behind the relation between these traits and CVD.

Since the associations between trait worry and elevated daytime and sleep HR are not mediated by states of worry or stress or biobehavioral variables, it remains unexplained which underlying psychological mechanism is responsible for the prolonged cardiac effects of worry. It is possible that a considerable part of the effect of trait worry is due to less conscious forms of perseverative cognition. At least three studies including our own suggest that a part of perseverative cognition may act in an unconscious fashion. Firstly, prolonged low HRV was found during sleep when anticipating a stressful oral speech that had to be performed after waking up (28). The second study showed prolonged cardiac effects during sleep following a day full of stress and worry (26). In the third study, worry showed prolonged cardiac effects up to 2 hours after the worry ended (24). Since in none of these cases conscious worry was possible (1,28) or reported (25), some other forms of perseverative cognition must have mediated these effects. The majority of cognitive processes operate without awareness, i.e. automatically (78, 79) and a considerable part of normal daily emotional processing - including PC - is likely to take place without us being aware (80). Hardly anything is known about the physiological effects of unconscious emotion, except for some brain and some skin conductance effects (81, 82), making this an important unexplored area of stress research.

The present study shows that incomplete recovery from neutral laboratory stressors is associated with elevated HR during daytime and sleep which is a most interesting finding. The literature shows that delayed cardiac recovery from cognitive (5-9) and physical (10-19) stressors is predictive of cardiac outcomes, such as hypertension, enhanced rest HR and blood pressure, abdominal adiposity, and even overall mortality ((5, 6, 8-11), reviewed in (20)). Our results suggest that a more incomplete recovery in the laboratory is associated with cardiac activity in daily life, more specifically elevated daytime and sleep HR. This is in line with results from Trivedi et al. (83) who found that blood pressure recovery in the laboratory predicted daytime and night-time ambulatory blood pressure levels after a work day. Moreover, Moseley and Linden (84) found that laboratory recovery even predicted ambulatory HR and blood pressure 3 years later. Apart from prospective disease risk and relations with physical factors, delayed cardiac recovery in the laboratory is found to relate to psychosocial factors as well. Delayed HR recovery after a treadmill test was observed in rehabilitated cardiac patients who reported an increased level of depressive symptoms (85). Additionally, there are some studies (86, 87) showing that low SES and delayed recovery are related. Others show evidence suggesting that high SES is a protective factor lowering the CVD risk that is induced by an incomplete recovery pattern after laboratory tasks (7, 88). Initially, cardiac recovery after neutral laboratory stressors was included in our analyses to account for individual basic recovery slopes, which are effected by physical characteristics of the individual including among others genetic makeup, fitness and BMI. However, the

effects of incomplete recovery in the laboratory on daytime and sleep HR were independent of age, gender, BMI and other biobehavioral factors measured on an hourly or subject level basis. Together with the findings in previous publications of a relation between delayed recovery and psychosocial factors (85, 86), this suggests that recovery from neutral laboratory stressors might reflect an autonomous factor, which is very likely to be low parasympathetic activity or low vagal tone (32, 89) activity. Rapid lowering of HR after exercise is thought to be accomplished by vagal reactivation after initial deactivation during exercise (90-93), which is why delayed cardiac recovery is seen as a marker for impaired autonomic nervous system functioning, more specifically impaired vagal tone. Apart from its physiological effects (94), vagal tone seems to be involved in the regulation of several psychological functions, such as anger control, attention, emotional regulation (95, 96). Additionally, findings from several studies seem to suggest that decreased depressive symptoms (85), higher SES (86) and improved physical fitness (13) are related to fast recovery and thus might improve impaired vagal functioning and eventually prevent serious health problems.

This study has several methodological limitations. We used the motility and cardiac data to detect and remove waking periods during sleep. There is recent evidence that subjects actually worry when they awaken at night (97). The exclusion of these awake periods from the analyses can possibly explain the lack of effects of worry after 10PM. On the other hand, deletion of awake periods seems to be an appropriate strategy if one is interested in the effects on cardiac levels during sleep only. Only polysomnographic data would have ensured us that all waking periods were deleted. As such, some of our limited effects on cardiac sleep levels might be due to awake periods instead of genuine prolonged effects during sleep. On the other hand, we expect that disturbances in sleep would be reflected in the reported level of sleep quality; statistically controlling for the effects of sleep quality did not change our results and therefore we do not expect that the inclusion of missed awake periods influenced our results. Yet, there is evidence that increased stress or worries before bedtime can increase the amount of awake time during sleep (29). Moreover, there is evidence that subjects actually worry when they awaken at night (97). Consequently, future studies that focus on the effects on physiological levels during actual sleep should be cautious to carefully assess whether participants are awake or not.

There are some additional methodological considerations concerning the measurement of cardiac recovery after neutral laboratory stressors. Firstly, one might argue that attenuated recovery in the laboratory might be influenced by anticipating the next task. Anticipating a stressor in the form of a laboratory task can induce physiological effects (98). However, to minimize these effects the tasks were presented in counterbalanced order. Secondly, the measurement of recovery was dependent on baseline and reactivity, and as such it was not a "clean" measurement of recovery. There is evidence that recovery after laboratory tasks is predictive of CVD outcome independent of reactivity to the same tasks (see for a review (20)). Our purpose was to capture an innate or typical stress curve during recovery, but not to find evidence for the explanatory power of recovery over reactivity or baseline; in our opinion, this can not be done without considering baseline and reactivity**.** Also, one might argue that the duration of this stress recovery period was too small (5 minutes). Since these tasks were relatively simple and of low impact, we

did not expect that a longer duration would be neccessary; also, when an individual does not recover within these 5 minutes this would be reflected in his AUC score. Moreover, Moseley & Linden (84) found that laboratory recovery with a duration of 5 minutes predicted ambulatory BP and HR levels.

In summary, the present study does not replicate cardiac effects of stressful events and worry during waking and nocturnal sleep. The results underscore the value of measuring the tendency to worry and the value of incomplete recovery from neutral laboratory stressors. Additionally, future ambulatory studies should consider the aspect of "thinking hard" when evaluating the effects of perseverative cognitions, since it might be crucially linked with toxic physiological changes.

Table 1: Mean, standard error, range and (positive) percentages for person level and day level variables.

	n	Mean ± SD	Range	%
Person level:				
Gender	55			70,9% Male
Age	55	46.1 ± 8.5	26 - 60	
BMI [a]	54	24.3 ± 3.5	17.2 – 34.1	
PSWQ [b]	55	43.0 ± 10.8	25 – 76	
WDQ [c]	55	22.0 ± 15.8	0 – 74	
BDI [d]	55	6.3 ± 5.5	0 – 24	
IHAT [e]	55	.19 ± .15	.0 - .67	
CM [f]	55	13.4 ± 6.0	3 – 27	
STAI [g]	55	36.4 ± 9.3	24 – 58	
Job strain [h]	55	-.28 ± 1.13	-2.8 – 5.2	
AUCHR	40	227.83 ± 31.67	158.41 – 301.31	
AUCMSSD	40	169.61 ± 32.36	89.15 – 277.20	
Day level:				
HR waking	149	79.80 ± 8.87	49.60 – 100.46	
HR sleep	149	61.79 ± 7.45	42.43 – 92.72	
MSSD waking	149	28.33 ± 11.80	6.52 – 72.20	
MSSD sleep	149	36.11 ± 19.20	6.70 – 126.79	
Frequency worry episodes waking	149	.08 ± .12	0 - .60	
Frequency stressful events waking	149	.11 ± .13	0 - .75	
Duration worry episodes (minutes) waking per entry	149	1.13 ± 3.15	0 – 30.50	
Duration stressful events (minutes) waking per entry	149	.75 ± 1.38	0 – 7.75	
Occurrence worry episodes after 10PM	143			21.7% Worry
Occurrence stressful events after 10PM	143			13.3% Stressful events
Duration worry episode (minutes) after 10PM if worry was reported	142	4.13 ± 11.73	0 - 60	
Duration stressful events (minutes) after 10PM if a	143	2.03 ± 8.07	0 - 60	

stressor was reported				
Type of day	143			52.4% Work
Coffee intake	147	.23 ± .20	0 – 3	
Smoking	148	.09 ± .26	0 - 3	
Alcohol intake	140	.11 ± .13	0 - 3	
Physical activity during waking	149	1.44 ± .34	1 - 5	
Resting during waking	149	1.19 ± .26	1 - 5	
% Physical activity during waking	149	.24 ± .12	0 - 1	
Sleep quality	143	2.85 ± .63	1 - 5	

[a] BDI=Body Mass Index
[b] PSWQ=Penn State Worry Questionnaire
[c] WDQ=Worry Domain Questionnaire
[d] BDI=Beck Depression Inventory
[e] IHAT= Interpersonal Hostility Assessment Technique
[f] CM=Cook-Medley Hostility Questionnaire
[g] STAI=Spielberger Trait Anxiety Inventory
[h] Job strain=high job demand, low control

Table 2: Effect of biobehavioral variables on cardiac activity during waking and sleeping (means and standard deviations)

		Waking		Sleeping	
		HR	MSSD	HR	MSSD
Gender					
	Male	79.52 ± 9.27	28.02 ± 12.27	60.95 ± 6.66	36.52 ± 18.71
	Female	80.65 ± 7.58	29.25 ± 10.35	64.32 ± 9.08	34.89 ± 20.82
Age					
	(<47.1 years)	80.57 ± 9.05	31.41 ± 13.80	60.65 ± 6.72	42.12 ± 22.50
	(>47.1 years)	79.09 ± 8.70	25.45 ± 8.71	62.85 ± 7.97	30.50 ± 13.36
BMI					
	(<24.3)	79.31 ± 9.21	31.19 ± 13.91	61.91 ± 8.55	39.83 ± 22.73
	(>24.3)	80.68 ± 8.40	25.14 ± 8.03	62.14 ± 5.89	31.84 ± 13.72
Coffee intake					
	No	76.19 ± 9.88	29.74 ± 14.38	58.39 ± 6.89	39.60 ± 19.30
	Yes	80.49 ± 8.57	28.06 ± 11.36	62.44 ± 7.36	35.46 ± 19.28
Smoking					
	No	79.01 ± 8.39	29.55 ± 11.49	60.55 ± 6.30	37.38 ± 18.76
	Yes	84.19 ± 10.55	21.60 ± 11.43	69.06 ± 9.74	29.59 ± 21.13
Alcohol intake					
	No	79.33 ± 8.43	28.55 ± 12.23	61.29 ± 7.27	37.09 ± 20.21
	Yes	80.63 ± 9.32	27.88 ± 11.88	62.44 ± 7.74	34.67 ± 18.98
Day					
	Work	81.50 ± 9.46	27.16 ± 10.93	62.46 ± 7.55	35.37 ± 17.96
	Leisure	78.54 ± 7.82	28.71 ± 11.36	61.50 ± 7.14	35.01 ± 16.73
Physical activity during waking					
	(<median)	79.23 ± 8.82	28.08 ± 10.79	62.30 ± 7.80	34.47 ± 19.09
	(>median)	80.35 ± 8.94	28.56 ± 12.77	61.30 ± 7.11	37.69 ± 19.29
Resting during waking					
	(<median)	80.72 ± 8.18	27.91 ± 9.82	62.98 ± 7.58	35.89 ± 17.03
	(>median)	78.96 ± 9.43	28.70 ±	60.69 ± 7.21	36.32 ±

		13.41		21.09
% Physical activity during waking				
(<median)	79.87 ± 8.83	29.03 ± 12.17	60.93 ± 8.32	37.41 ± 20.70
(>median)	79.73 ± 8.97	27.63 ± 11.46	62.63 ± 6.43	34.84 ± 17.63

Table 3: Correlations between worry and stressor variables, trait values and physical activity and cardiac activity during waking and sleeping

	Waking		Sleeping	
	HR	MSSD	HR	MSSD
Stressor frequency waking	.18	.03	.05	.17
Stressor duration waking	.32*	-.08	.30*	.04
Worry frequency waking	-.02	.14	.02	.06
Worry duration waking	.09	.14	.17	-.02
Stressor occurrence during the night	.02	.15	-.06	.21
Stressor duration during the night	.05	.13	.00	.16
Worry occurrence sleep	.09	.07	.05	-.02
Worry duration sleep	.15	-.04	.13	-.11
Hostility (CM) [a]	.03	-.15	.03	-.02
Hostility (IHAT) [b]	.09	-.30*	.21	-.25
Depression (BDI) [c]	-.23	-.11	-.03	-.05
Anxiety (STAI)[d]	.01	-.06	.12	-.03
Worry (PSWQ) [e]	.15	-.15	.30*	-.15
Worry (WDQ) [f]	-.11	-.06	.04	-.08
Job strain [g]	.01	.24	-.14	.25
AUC recovery HR	.56**		.36*	
AUC recovery MSSD		.28		.09

[a] CM=Cook-Medley Hostility Questionnaire
[b] IHAT= Interpersonal Hostility Assessment Technique
[c] BDI=Beck Depression Inventory
[d] STAI=Spielberger Trait Anxiety Inventory
[e] PSWQ=Penn State Worry Questionnaire
[f] WDQ=Worry Domain Questionnaire
[g] Job strain=high job demand, low control

Table 4: Effects of frequency and duration of stressful events and worry episodes on HR and lnMSSD during waking.

	HR		lnMSSD	
	Frequency	Duration	Frequency	Duration
Fixed effects				
Intercept	75.63 ± 1.10	7.53 ± 1.10	3.42 ± .05	3.43 ± .05
Stressor frequency	-1.22 ± 5.77		-.08 ± .26	
Worry frequency	.96 ± 5.30		-.07 ± .24	
Stressor duration		-.62 ± 1.06		.02 ± .05
Worry duration		.68 ± .76		-.00 ± .03
Smoking	5.76 ± 4.98	6.24 ± 5.05	-.32 ± .23	-.32 ± .23
Alcohol consumption	-.98 ± 3.84	-.83 ± 3.79	.25 ± .18	.26 ± .18
Coffee consumption	-7.78 ± 4.76	-7.76 ± 4.71	.40 ± .22	.40 ± .22
Gender	3.15 ± 1.82*	3.02 ± 1.83*	-.06 ± .09	-.06 ± .09
Age	-.17 ± .11	-.16 ± .12	-.01 ± .005*	-.01 ± .005*
BMI [a]	-.28 ± .27	-.27 ± .27	-.01 ± .01	-.01 ± .01
Type of day	4.11 ± 1.01**	4.18 ± 1.01**	-.06 ± .05	-.07 ± .05
% High activity	-4.10 ± 4.14	-4.43 ± 4.08	.16 ± .19	.20 ± .19
Activity level	17.78 ± 4.60**	17.86 ± 4.51**	-.18 ± .21	-.16 ± .21
Resting during awake	-1.09 ± 4.83	-.93 ± 4.76	-.08 ± .22	-.06 ± .22
Hostility (CM) [b]	-.19 ± .24	-.23 ± .24	.02 ± .01	.02 ± .01
Hostility (IHAT) [c]	7.64 ± 7.00	7.60 ± 7.03	-.45 ± .32	-.43 ± .32
Depression (BDI) [d]	-.13 ± .22	-.13 ± .22	-.00 ± .01	-.00 ± .01
Anxiety (STAI)[e]	-.13 ± .17	-.13 ± .17	-.02 ± .01*	-.02 ± .01*
Worry (PSWQ) [f]	.26 ± .13*	.26 ± .13*	.00 ± .01	.00 ± .01
Worry (WDQ) [g]	.00 ± .06	-.01 ± .06	.00 ± .00	.00 ± .00
Job strain [h]	-.30 ± .70	-.31 ± .70	.02 ± .03	.02 ± .03
AUCHR	.09 ± .03**	.10 ± .03**		
AUCMSSD			.003 ± .001	.003 ± .001
Variance components				
Person level:				
Intercept (σ^2_u)	12.93 ± 4.46	13.41 ± 4.53	.03 ± .01	.03 ± .01
Episode level:				
Intercept (σ^2_e)	13.76 ± 2.50	13.41 ± 2.44	.03 ± .01	.03 ± .01
Deviance	573.99	573.07	-24.37	-24.27

[a] BMI=Body Mass Index

[b] CM=Cook-Medley Hostility Questionnaire

[c] IHAT= Interpersonal Hostility Assessment Technique
[d] BDI=Beck Depression Inventory
[e] STAI=Spielberger Trait Anxiety Inventory
[f] PSWQ=Penn State Worry Questionnaire
[g] WDQ=Worry Domain Questionnaire
[h] Job strain=high job demand, low control
** p<.01 based on one-tailed t-tests
* p<.05 based on one-tailed t-tests

Table 5: Effects of frequency and duration of stressful events and worry episodes on HR and lnMSSD during sleep.

	HR		lnMSSD	
	Frequency	Duration	Frequency	Duration
Fixed effects				
Intercept	59.14 ± .92**	59.34 ± .97**	3.62 ± .08**	3.61 ± .08**
Stressor frequency waking	-10.47 ± 5.46		.50 ± .33+	
Worry frequency waking	7.70 ± 5.46		-.38 ± .32	
Stressor frequency night	3.31 ± 2.11		-.02 ± .13	
Worry frequency night	-1.63 ± 1.50		.05 ± .09	
Stressor duration waking		.00 ± 1.01		.05 ± .06
Worry duration waking		1.14 ± .76		-.07 ± .05+
Stressor duration night		1.32 ± 1.22		-.00 ± .07
Worry duration night		-.93 ± 1.00		.02 ± .06
Smoking	5.93 ± 4.25	4.94 ± 4.60	.73 ± .35*	.69 ± .35+
Alcohol consumption	8.74 ± 3.62**	9.72 ± 3.61**	-.47 ± .22*	-.52 ± .22**
Coffee consumption	-.72 ± 4.42	1.18 ± 4.52	-.39 ± .29	-.44 ± .29
Gender	3.86 ± 1.46**	3.90 ± 1.59**	1.15 ± .13	-.15 ± .14
Age	.02 ± .09	.01 ± .10	-.02 ± .01	-.02 ± .01
BMI [a]	-.33 ± .22	-.27 ± .23	-.01 ± .02	-.01 ± .02
Type of day	1.45 ± .99	.90 ± .98	-.07 ± .06	-.05 ± .06
% High activity	1.81 ± 4.10	3.55 ± 4.02	-.22 ± .25	-.26 ± .24
Activity level	-1.56 ± 4.21	-.05 ± 4.18	-.08 ± .26	-.12 ± .26
Resting during awake	-3.38 ± 4.37	-1.07 ± 4.35	-.42 ± .27	-.49 ± .27
Sleep quality	-.70 ± .73	-.61 ± .71	.07 ± .04*	.06 ± .04*
Hostility (CM) [b]	-.32 ± .19	-.28 ± .21	.01 ± .02	.01 ± .02
Hostility (IHAT) [c]	7.84 ± 5.62	9.19 ± 6.10	-.37 ± .50	-.42 ± .51
Depression (BDI) [d]	-.12 ± .18	-.05 ± .19	.00 ± .02	.00 ± .02
Anxiety	.05 ± .15	-.02 ± .15	-.01 ± .01	-.01 ± .01

(STAI)[e]				
Worry (PSWQ)[f]	.18 ± .10*	.14 ± .11	.01 ± .01	.01 ± .01
Worry (WDQ)[g]	.04 ± .05	.06 ± .05	-.00 ± .00	-.00 ± .00
Job strain[h]	-.75 ± .57	-.56 ± .61	.03 ± .05	.02 ± .05
AUCHR	.06 ± .02**	.07 ± .03**		
AUCMSSD			.00 ± .00	.00 ± .00
Variance components				
Person level:				
Intercept (σ^2_u)	5.92 ± 2.74	8.76 ± 3.30	.09 ± .03	.09 ± .03
Episode level:				
Intercept (σ^2_e)	12.76 ± 2.34	11.69 ± 2.15	.04 ± .01	.04 ± .01
Deviance	539.64	541.50	25.48	25.30

[a] BMI=Body Mass Index
[b] CM=Cook-Medley Hostility Questionnaire
[c] IHAT= Interpersonal Hostility Assessment Technique
[d] BDI=Beck Depression Inventory
[e] STAI=Spielberger Trait Anxiety Inventory
[f] PSWQ=Penn State Worry Questionnaire
[g] WDQ=Worry Domain Questionnaire
[h] Job strain=high job demand, low control** p<.01 based on one-tailed t-tests
** p<.01 based on one-tailed t-tests
* p<.05 based on one-tailed t-tests

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