

Associations between the cortisol awakening response and heart rate variability

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Summary The process of morning awakening is associated with a marked increase in cortisol secretion, the cortisol awakening response (CAR), as well as with a burst in cardiovascular (CV) activation. Whilst the CAR is largely driven by awakening-induced activation of hypothalamic–pituitary–adrenal axis, it is fine-tuned by direct sympathetic input to the adrenal gland. In parallel, awakening-induced activation of the CV system is associated with a shift towards dominance of the sympathetic branch of the autonomic nervous system. Moreover, the CAR, in common with trait-like heart rate variability (HRV), is widely reported to be associated with psychosocial variables and health outcomes. These commonalities led us to examine associations between the CAR and both concurrent awakening-induced changes and trait-like estimates in cardiovascular activity (heart rate (HR) and HRV). Self-report measures of difficulties in emotion regulation and chronic stress were also obtained. Forty-three healthy participants (mean age: 23 years) were examined on two consecutive weekdays. On both days, heart interbeat interval (IBI) data was obtained from sedentary laboratory recordings as well as from recordings over the peri-awakening period. Salivary free cortisol concentrations were determined on awakening and 15, 30, and 45 min post-awakening on both study days. Data from a minimum of 36 participants were available for individual analyses. Results revealed significant awakening-induced changes in cortisol, HR and HRV measures; however, no associations were found between the simultaneous post-awakening changes of these variables. Similarly, awakening-induced changes in cortisol, HR and HRV measures were not significantly associated with perceived stress or measures of emotion regulation. However, the CAR was found to be significantly positively correlated with steady state measures of HR and negatively correlated with steady state measures of HRV, as determined during the laboratory sessions and the peri-awakening periods. This cross-sectional study indicates that, despite consistent associations between the CAR and indices of trait-like cardiovascular activity, the CAR is not related to concurrent changes of cardiac autonomic activation following awakening.

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Introduction

Secretion of the hormone cortisol is characterised by a marked circadian rhythmicity. Within this circadian rhythm, the process of morning awakening provides a distinct stimulatory influence associated with a marked increase in cortisol levels over the first 30–45 min following awakening: the cortisol awakening response (CAR; Pruessner et al., 1997; Wilhelm et al., 2007). Evidence suggests that the CAR is a product of complex regulatory processes, including awakening-induced activation of the hypothalamic–pituitary–adrenal (HPA) axis as well as fine-tuning by direct neural input to the adrenal gland that modulates adrenal sensitivity to ACTH during the peri-awakening period (see Clow et al., *in press* for a review). These changes in adrenal sensitivity to ACTH are regulated by the hypothalamic suprachiasmatic nucleus (SCN; Buijs et al., 1997, 2003; Bornstein et al., 2008) and mediated via sympathetic innervation of the adrenal gland by the splanchnic nerve (Edwards and Jones, 1993; Ehrhart-Bornstein et al., 1998; Sage et al., 2002; Engeland and Arnhold, 2005; Ulrich-Lai et al., 2006). Over the past decade, the CAR has become a frequently examined parameter in psychobiological research and associations between the CAR and a wide range of variables in the psychosocial and health domains have been reported (see Clow et al., 2004; Fries et al., 2009). Whilst this has led to some level of understanding with regard to psychological correlates of the CAR, to date there has been limited investigation into its broader physiological correlates and how it relates to parallel awakening-induced physiological responses in particular.

In addition to the HPA axis, the autonomic nervous system (ANS) forms a second major physiological response system that affects almost all bodily functions and plays a pivotal role in normal and stress-responsive physiology and the maintenance of homeostasis (Jänig, 2006). The HPA axis and ANS are known to be integrated at several levels (e.g. see Evans et al., 2000; Tafet and Bernardini, 2003) and are increasingly studied together (e.g. Licht et al., 2010). Furthermore, both the HPA axis and ANS show marked circadian rhythmicity (e.g. see Kräuchi, 2002; Hermida et al., 2007; Rasch et al., 2007). Specific influences of the process of awakening have also been reported for the ANS-controlled cardiovascular variables of heart rate (HR) and heart rate variability (HRV; below summarised as 'HR/HRV'). Huikuri et al. (1994) found that mean HR increased as a response to morning awakening and that this increase occurs in the absence of postural changes from a supine to an upright position. Similarly, in the absence of postural changes, awakening was associated with an increase in low frequency (LF) HRV and the LF/high frequency (HF) ratio as well as with a decrease in HF HRV levels (Huikuri et al., 1994). Since HF HRV is considered to be predominantly mediated by input of the parasympathetic branch of the ANS whilst the LF/HF ratio is seen as an index of sympathovagal balance (Task Force, 1996), these findings suggest that awakening is associated with a shift in the balance of cardiac ANS input towards sympathetic dominance.

The fact that awakening is associated with both changes in cortisol secretion and HR/HRV and that both have been linked with activation of the sympathetic nervous system poses the interesting question whether these awakening-induced changes are related to each other. Besides their common

initiation by the process of awakening, the possibility of a relationship between the CAR and HR awakening changes is also suggested by findings of morning-specific influences of light on cortisol secretion (Leprout et al., 2001) and light-induced effects on the CAR (Scheer and Buijs, 1999; Thorn et al., 2004), with particularly the latter implicating the SCN in the regulation of the CAR (Clow et al., 2004, *in press*). Intriguingly, analogous results have been reported for HR levels, which also show marked increases as a result of light exposure (Scheer et al., 1999, 2001; Saito et al., 1996) that are confined to the morning period (Scheer et al., 1999, 2004). Combining these sets of findings with neuroanatomical evidence, Scheer et al. (2003) have suggested that light-induced influences on both cortisol secretion and HR might be mediated by a common SCN-dependent neuronal pathway via the sympathetic nervous system (Scheer et al., 2003).

In addition, these two sets of awakening changes might also be functionally related. It has been hypothesised that the CAR plays an activating role, providing the organism with the 'energetic boost' required for the diurnal phase of activity (Pruessner et al., 1997; Adam et al., 2006). Findings of positive associations between the magnitude of the CAR and state arousal 45 min post-awakening (e.g. Thorn et al., 2004, 2009; Stalder et al., 2010) are consistent with this notion. Similarly, an increase in HR and the associated shift in sympathovagal balance towards sympathetic dominance, leading to enhanced oxygen supply to the organs and preparation for increased activity (e.g. Berne and Levy, 1998; Jänig, 2006), is clearly related to physiological activation. Together, the assumed commonality of regulatory input and physiological role points to a potential relationship between the CAR and awakening-induced HR/HRV changes and examining their relationship provides an opportunity to extend the knowledge about physiological correlates of the CAR.

In addition to an examination of acute awakening-induced changes, another line of evidence might suggest that an investigation into more trait-like associations between these variables is also of value. Both the CAR and HRV have been considered as trait biological markers for variables in the psychosocial domain. Whilst findings with regard to the CAR have been characterised by some inconsistency (see Fries et al., 2009), a recent meta-analysis suggests that across studies the CAR tends to be positively associated with indices of stress and inversely associated with fatigue, burnout or exhaustion (Chida and Steptoe, 2009). Similarly, alterations of HRV have also been found to be associated with acute (Hall et al., 2004) and chronic stress (Lucini et al., 2005; Vrijkotte et al., 2000). In addition, two independent theories, the *model of neurovisceral integration* (Thayer and Lane, 2000, 2009) and the *polyvagal theory* (Porges, 1997, 2001), have proposed a link between parasympathetically mediated HRV and regulated emotional responding—a broad ability assumed to be vital for social functioning and the maintenance of mental health (Gross and Muñoz, 1995; Gross, 1998). Together this points to a general overlap in the assumed marker qualities of the CAR and indexes of HRV, suggesting that trait estimates of these variables might be related.

One aim of the current study was to examine relationships between the CAR and concurrent awakening-induced changes in HR/HRV measures. Based on the reviewed evidence, a positive association between the CAR and awakening changes in HR as well as HRV indexes of sympathovagal

balance is predicted. In addition the study aimed to examine more trait-like associations between the CAR and HR/HRV measures. For this, HR/HRV estimates from controlled sedentary laboratory recordings as well as from the peri-awakening period were examined. In addition, to aid the interpretation of physiological results, self-report measures of chronic stress and dysfunction in emotion regulation were examined for associations with the CAR and HR/HRV estimates.

Methods

Participants and recruitment

Participants were 43 healthy psychology students from the University of Westminster. Demographic data was available for only 38 students as five students did not return the respective material. Of the 38 who provided data, 10 were male and 28 female and participants' mean age was 23 years (range: 18–40 years). Ten participants described themselves as smokers but agreed to refrain from smoking over the post-awakening periods of the study days. The protocol of the study was approved by the ethics committee of the University of Westminster and all participants provided written informed consent.

Design and procedure

The study was carried out on two consecutive weekdays for each participant. The identical protocol for each study day is illustrated in Fig. 1. The first part of each day took place at the University of Westminster. During this session participants received detailed instructions about the study and were shown how to attach the heart interbeat interval (IBI) monitor (Actiheart; see below for details) to their chest area using two ECG electrodes. A test of the IBI signal strength was then carried out and, when successful, this was followed by a 13 min laboratory IBI recording (explained in detail below). Subsequently, participants were allowed to take off the Actiheart monitor whilst keeping the ECG electrodes attached to their chest. They left the laboratory and the second part of the study (peri-awakening) was carried out at their domestic settings.

Before going to bed on the evenings following laboratory recordings, participants re-attached the Actiheart monitor to the electrodes. IBI and motility data was then recorded over night and the first 45 min post-awakening on each study day. Following awakening, participants were told to sit up in their beds and to move as little as possible for the following 15 min to provide a more controlled IBI recording. At 15 min

post-awakening, participants were allowed to get up and move around freely. Saliva samples were taken immediately on awakening and at 15, 30, and 45 min post-awakening on each study day. During this period participants were instructed not to take anything by mouth, other than water, or to brush their teeth to avoid abrasion and micro-vascular leakage. Participants were asked to record their time of awakening and saliva sampling times on a recording table. Participants returned to the University laboratory the following day and the complete procedure was repeated.

HR/HRV and motility data

Materials and measurement

Actiheart monitors (Cambridge Neurotechnology, Cambridge, UK) were used to record motility as well as heart interbeat interval (IBI) data. These are flat and lightweight (<10 g) devices for which high levels of intra- and inter-instrument reliability as well as good validity of measures have been reported (Brage et al., 2005). Simultaneous motility and heart rate readings from Actiheart monitors were also used as an additional objective check on awakening time in the current study, as previously described by Stalder et al. (2009).

Following preparation of respective skin areas using standard cleaning pads and *Unilect 2121M* abrasive pads (Unomedical Ltd., Redditch, UK), Actiheart monitors were attached to the chest area using two *Unilect 4040M* ECG electrodes (Unomedical Ltd., Redditch, UK). Electrodes were placed below the sternum (mid-way below the V1 and V2 positions) and horizontally towards the left side of the body from this position. A signal test was then performed using *Actiheart 2.132* software (Cambridge Neurotechnology, Cambridge, UK).

For HR/HRV analysis, IBI data was read in using *Actiheart software 2.132* and edited in a two-step procedure: firstly, data was edited using the automated editing programme of the software, followed by manual inspection of the complete IBI time series and the removal of remaining artefacts. Following editing, IBI data was transferred to *HRV Analysis 1.1 SPI* software (Biomedical Signal Analysis Group, University of Kuopio, Finland) for the calculation of HR/HRV parameters. Five measures were derived from IBI data. Mean heart rate was calculated as heart beats per minute and the *standard deviation of normal-to-normal intervals (SDNN)* was calculated as a time domain measure of overall heart rate variability. Three non-parametric frequency domain measures were further calculated in the form of *low frequency (LF)*; .04–.015 Hz and *high frequency (HF)*; .15–.4 Hz HRV as well as the LF/HF ratio (Task Force, 1996).

Laboratory recordings

For the laboratory recording, participants sat on a chair with their eyes closed and their arms in a resting position. They were asked to move as little as possible and to breathe normally. For the recording, participants wore headphones over which they listened to a standardised commentary which repeated the above instructions. The recording period followed and consisted of a silent period of 6 min followed by another 6 min period during which participants listened to natural environmental sounds (such as running water, etc.).

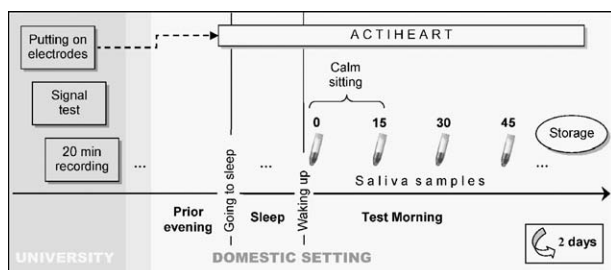


Figure 1 Overview of the protocol for each study day.

For each laboratory recording two time periods were examined, which covered 5 min from the silence- and the natural sounds period, respectively. HR and HRV measures of the two recording periods were highly correlated (mean $r = .79$; range: .50–.96) and thus mean values of the two recording periods were calculated and used in further analyses. These mean values were also highly correlated between laboratory recordings on the two study days (mean $r = .65$; range: .42–.81); hence, mean data of the two laboratory recordings were calculated and used in statistical analyses.

Peri-awakening recordings

The aim of these recordings was to allow a detailed examination of HR/HRV dynamics from the period 1 h pre-awakening to 45 min post-awakening. Three main factors had to be considered: (i) to allow adequate examination of associations between HR/HRV and cortisol data and to obtain detailed descriptive information of HR/HRV dynamics over the pre- and post-awakening period, the overall time period had to be divided into shorter sub-periods. (ii) Since arousing from sleep is known to be associated with a rapid cardiovascular activating response lasting approximately 10–15 heartbeats (Trinder et al., 2001, 2003), it was also important to recess the minute around awakening from the analysis to avoid imposing an error on HR/HRV results. (iii) Finally, quantification periods needed to be of equal length, since estimates of HRV parameters are influenced by the length of examined time periods (Task Force, 1996).

Based on these considerations, four sub-periods (PRE, POST1, POST2, and POST3), each made up of a number of 4 min 40 s quantification periods were employed. Fig. 2 illustrates this strategy in relation to the saliva sampling times. This measurement strategy satisfied the above criteria since sub-periods can be used for intra- and inter-individual analyses whilst the quantification periods allow provision of detailed descriptive information.

High levels of stability were found across the two study days for HR/HRV measures for the peri-awakening sub-periods (mean $r = .73$, range: .25–.89). Thus, mean data from the two sets of recordings was calculated and used in statistical analyses.

Psychosocial measures

The 14-item Perceived Stress Scale (PSS; Cohen et al., 1983) was used to measure perceived stress over the previous

month. Items were measured on a five-point scale and scores could range from 0 to 56, with higher scores indicating higher perceived stress. The Difficulties in Emotion Regulation Scale (DERS; Gratz and Roemer, 2004) was used to assess difficulties in emotion regulation. Whilst this 36-item measures assesses typical levels of emotion dysregulation across six domains, in the current study only results of the total DERS score (range of scores: 36–180), reflecting overall emotion dysregulation, are reported.

Data exclusion and statistical analysis

One participant provided insufficient saliva for assaying on both study days, whilst for two participants this was the case on one of the two study days. Data were excluded if the participant self-reported a delay >10 min in taking any of the saliva samples or if a difference >10 min between self-reported awakening time and the objective proxy of awakening time (based on combined motility and HR readings) was found. Based on these criteria, data of eight further study days (five on day 1) were excluded from analyses, leaving 36 full sets of cortisol data for the first study day and 38 sets for the second study day. Heart IBI data of ambulatory recordings were not available from three participants on the first study day and from two participants on the second study day due to signal loss during the night.

Cortisol data were square root transformed to reduce positive skewness. Repeated-measures ANOVAs were performed separately for the two study days to examine changes in cortisol concentrations over the post-awakening period, i.e. the CAR. Two measures were calculated to quantify post-awakening cortisol levels: the level of cortisol on awakening (S1), which describes the end-point of the pre-awakening cortisol increase. The area under the cortisol curve with respect to increase (AUC_i; Pruessner et al., 2003) was used as a measure for the CAR. Associations of these measures across days were examined using a correlation analysis.

Changes in HR/HRV measures and motility levels over the awakening period were also examined using repeated-measures ANOVAs on data of the PRE, POST1, POST2 and POST3 sub-periods. Following findings of significant main effects of time, differences between sub-periods were examined using post hoc analyses with Bonferroni-corrected significance probabilities. Significance probabilities for within-days terms in all ANOVAs were corrected for sphericity violations where appropriate using the Greenhouse–Geisser method.

Associations between cortisol and HR/HRV measures of laboratory and peri-awakening recording periods were examined using correlation analyses. Results of associations with HR/HRV measures of peri-awakening recordings were confirmed in partial correlation analyses controlling for time of awakening and the respective motility level. Similarly, associations between cortisol measures and awakening changes in HR/HRV measures as well as those between cortisol and HR/HRV measures and psychosocial measures were examined using correlation analyses and, where appropriate, confirmed in partial correlation analyses controlling for time of awakening and motility levels.

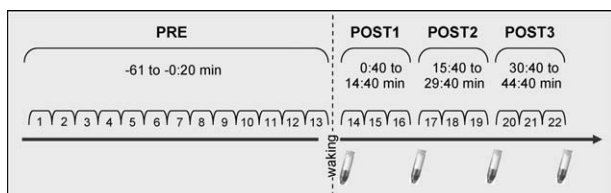


Figure 2 The four sub-periods (PRE and POST1–3) used for quantification of HR/HRV measures and the shorter quantification periods that make up these periods. Each of the 22 quantification periods lasted 4 min 40 s. HR/HRV periods are shown in relation to saliva sampling times; awakening indicated by the perpendicular dashed line.

Results

Cortisol data

Fig. 3 illustrates mean post-awakening cortisol levels separately for the two study days. The ANOVAs of changes in cortisol values over the post-awakening period, carried out separately for both study days, showed a significant effect of sampling time on both days (day 1: $F_{[1.5, 54.2]} = 40.95$, $p < .001$; day 2: $F_{[1.6, 59.3]} = 41.70$, $p < .001$), demonstrating the cortisol awakening response as expected. An awakening response of less than 2.5 nmol/L cortisol, thought to comprise a secretory episode (Wüst et al., 2000), was seen on 15.8% of study days prior to exclusion of suspected non-adherers and 10.8% of days after exclusion. Levels of S1 ($r = .68$, $p < .001$) and the AUC_i ($r = .52$, $p = .002$) were positively correlated across days and thus the mean across both sampling days was used in all subsequent analyses. Participants' mean (range) awakening times were 07:05 h (03:15–9:43 h) on day 1 and 07:36 h (04:42–10:28 h) on day 2. Awakening time was found to be inversely related to the AUC_i on both day 1 ($r = -.36$, $p = .03$) and day 2 ($r = -.38$, $p = .02$).

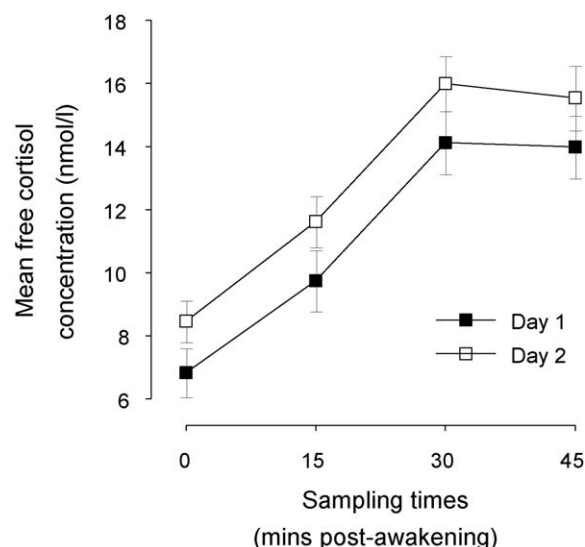


Figure 3 Mean (\pm standard error of the mean; SEM) free cortisol concentrations (nmol/L) for study day 1 ($N = 36$) and study day 2 ($N = 38$).

Heart rate and heart rate variability data

Fig. 4 displays two-day mean (\pm standard error of the mean; SEM) HR and HRV data for the sedentary laboratory recordings

as well as for the 22 quantification periods of the peri-awakening recordings.

The repeated-measures ANOVAs of within-day changes in HR/HRV indexes over the four peri-awakening sub-periods

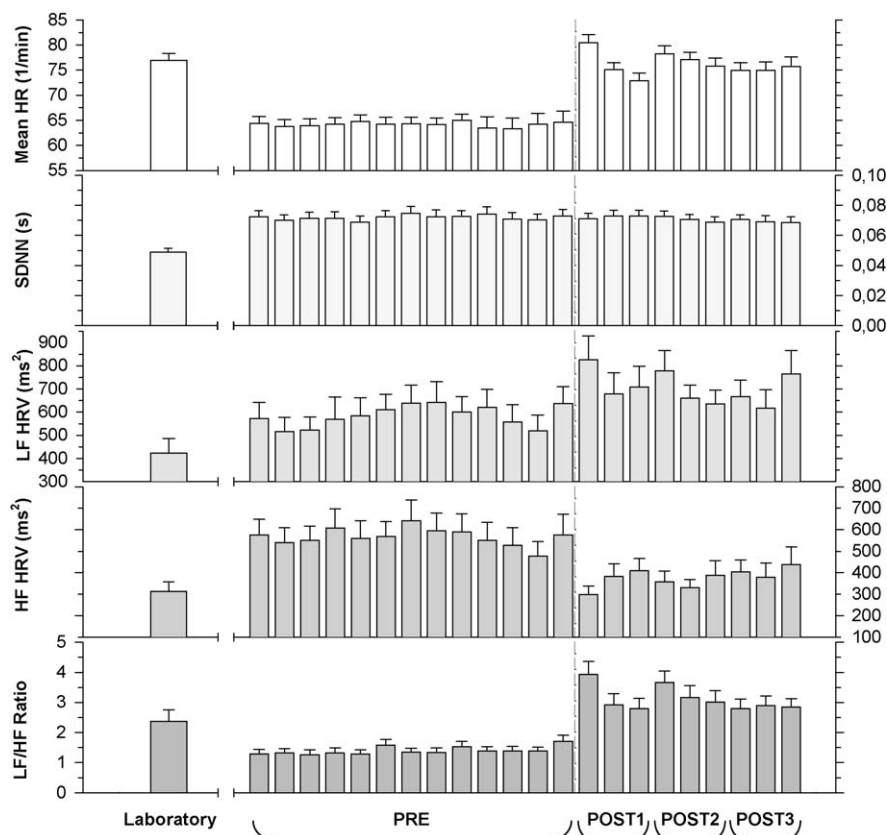


Figure 4 Two-day mean HR, SDNN, LF HRV, HF HRV and LF/HF ratio values for laboratory recordings ($N = 41$) and for the 22 peri-awakening quantification periods ($N = 38$). Error bars represent the standard error of the mean (SEM). Awakening is indicated by the dashed line.

revealed significant effects of time for mean HR ($F_{(2.2,88.9)} = 46.63$, $p < .001$), HF HRV ($F_{(2.3,92.8)} = 11.21$, $p < .001$) and LF/HF ratio ($F_{(2.5,101.0)} = 16.19$, $p < .001$), whilst no significant effects of time were found for SDNN ($F_{(2.5,88.7)} = .67$, n.s.) or LF HRV ($F_{(2.5,103.7)} = 1.09$, n.s.). Post hoc analyses revealed: (i) mean HR and LF/HF ratios were lower during the PRE than during all POST-awakening periods ($'p's < .001$) and (ii) HF HRV PRE levels were higher than during all POST-awakening periods ($p < .001$). Awakening time and motility levels of peri-awakening recording periods were not found to be significantly related to HR/HRV measures of the respective periods (all $'p's > .05$).

Since no significant differences were found between the three POST sub-periods for any of the HR/HRV variables (all $'p's > .3$), these sub-periods were collapsed into a single POST period, which was used in subsequent analyses.

Relationships between laboratory and peri-awakening HR/HRV measures

Two-day mean HR, SDNN, LF and HF HRV measures from the sedentary laboratory sessions were highly positively correlated with two-day mean steady state measures from the PRE (mean $r = .56$, range: $.39-.68$) and the POST (mean $r = .50$, range: $.30-.60$) recording periods. Two-day mean LF/HF ratio laboratory recordings were not significantly related to respective steady state mean PRE ($r = -.16$, n.s.) or POST ($r = .29$, $p = .08$) recordings.

Measures of awakening-induced changes were only calculated for mean HR, HF HRV and the LF/HF ratio since only these variables were found to change significantly with awakening. Awakening-induced changes in each variable were calculated as the change from the PRE-awakening period to the complete POST-awakening period (change: POST–PRE). Awakening-induced changes in HR/HRV measures were highly correlated across days (mean $r = .75$, range: $.60-.82$) and thus two-day mean values were used in subsequent analyses.

An examination of relationships between two-day mean laboratory and awakening-induced change measures revealed an inverse relationship between two-day laboratory recordings and awakening-induced changes in HF HRV ($r = -.49$, $p = .002$), whilst no significant relationships between laboratory and awakening-induced measures were found for other HR/HRV variables (all $'p's > .2$).

Post-awakening cortisol and HR/HRV measures

No significant associations were found between awakening-induced changes in HR, HF HRV and the LF/HF ratio and either S1 or the AUC_I (all $'p's > .05$). Controlling for time of awakening or respective awakening changes in motility levels did not alter these results.

Table 1 provides data on associations between the 2-day mean of cortisol measures and laboratory and peri-awakening HR/HRV data. S1 was not significantly related to any HR/HRV measure; however, the AUC_I was found to be positively related to laboratory levels of mean HR and inversely related to those of SDNN, LF HRV and HF HRV. In addition, these associations were confirmed by respective data from both peri-awakening PRE and POST recordings. Whilst controlling for awakening times or respective motility levels marginally

Table 1 Relationships between two-day mean cortisol measures and HR/HRV measures from laboratory and peri-awakening recordings.

HR/HRV		Cortisol	
Measure	Recording	S1	AUC _I
Mean HR	Laboratory	-.04	.32*
	Amb. PRE	.06	.35*
	Amb. POST	-.00	.31*
	PRE to POST change	-.08	.02
SDNN	Laboratory	.25	-.45**
	Amb. PRE	.27	-.43**
	Amb. POST	.12	-.44**
	PRE to POST change	-.03	.23
LF	Laboratory	.09	-.36*
	Amb. PRE	.21	-.30†
	Amb. POST	.18	-.40*
	PRE to POST change	-.03	-.16
HF	Laboratory	.23	-.31*
	Amb. PRE	.22	-.30†
	Amb. POST	.08	-.34*
	PRE to POST change	-.24	.11
LF/HF ratio	Laboratory	-.13	-.17
	Amb. PRE	.12	-.20
	Amb. POST	-.03	-.05
	PRE to POST change	-.09	.05

** $p \leq .01$ (2-tailed).

* $p \leq .05$ (2-tailed).

† $p \leq .10$ (2-tailed).

altered the strength of some the associations between the AUC_I and ambulatory HR/HRV measures to non-significant trends, the overall pattern of results remained unchanged.

Associations with psychosocial variables

Participants' mean scores for the DERS were 79.2 (SD = 20.1, range: 50–138), while mean scores for the PSS were 22.7 (SD = 7.1, range: 12–39). No significant associations were found between the DERS and the two-day mean of S1 ($r = -.04$, $p = .83$) or the AUC_I ($r = .22$, $p = .21$). Similarly the PSS was not significantly related to the two-day mean of S1 ($r = .05$, $p = .78$); however, a non-significant trend for a positive relationship between this measure and the two-day mean of the AUC_I was found ($r = .29$, $p = .086$).

No significant associations between psychosocial measures and laboratory HRV recordings were found. However, highly significant positive relationships were found between mean HR and the DERS ($r = .59$, $p < .001$) as well as the PSS ($r = .63$, $p < .001$). Correlation analyses of two-day mean awakening changes in HR/HRV measures revealed no significant associations with psychosocial measures.

Discussion

In the current study we set out to examine associations between the CAR, and HR/HRV recordings ascertained from

the peri-awakening period and sedentary laboratory sessions. Whilst clear changes with awakening were found for cortisol and HR/HRV measures, the hypothesis of a relationship between these awakening changes was not confirmed by the current data. On the other hand, the present findings provide evidence for an association between the CAR and trait-like estimates of HR/HRV measures from both laboratory and peri-awakening period recordings, suggesting that individuals who exhibit a more elevated CAR typically show higher levels of HR as well as lower levels of overall HRV.

The rationale for the current study was based on evidence indicating that the process of awakening is associated with concurrent changes in both cortisol secretion and HR/HRV, with the possibility of some shared regulatory input from the sympathetic nervous system. On a descriptive level, the findings confirm previous evidence insofar as clear and consistent awakening-induced changes in cortisol levels as well as in mean HR, HF HRV and the LF/HF ratio were seen. The latter findings thus parallel results of [Huikuri et al. \(1994\)](#), with the exception that in the present study no significant awakening-induced increase in LF HRV was observed. However, no evidence was seen that concurrent awakening-induced changes in cortisol levels and HR/HRV measures were related to each other. Another noteworthy difference between these awakening-induced responses was that the CAR was (as often recorded) inversely correlated with time of awakening whereas the change in cardiovascular measures was not. Whilst this study does not rule out a role of autonomic activation in the 'fine-tuning' of the CAR, it was unable to provide supportive evidence by examination of simultaneous changes in CV function. It is apparent that the CAR is a complex synergistic measure involving activation of the HPA axis ([Wilhelm et al., 2007](#)) as well as of the autonomic nervous system ([Clow et al., in press](#)). As this study did not measure levels of available ACTH pre- and post-awakening, it was unable to take account of the contribution of individual differences in HPA axis status and activation, which no doubt contributed greatly to the between-participant variance in the CAR. It is interesting to note here that dissociations between activation of the HPA axis and the ANS after stress have also been reported ([Schommer et al., 2003](#)). An additional rationale for this study was based on the notion that the CAR and awakening-induced HR/HRV changes might both fulfil a role in providing the organism with the energy required for shifting from a resting (nocturnal) to an active (diurnal) phase ([Pruessner et al., 1997](#)). Despite the lack of a close association between the examined measures, it remains possible that awakening-induced activation of both physiological systems perform this role, as their temporal relationship is consistent with shared functionality, even if they arise from different regulatory systems.

An interesting observation from the current study was that awakening-induced changes in autonomic function were (apart from HF HRV) not related to steady state measures. In other words, trait-like measures of cardiovascular activity determined during a sedentary laboratory session, were not associated with the cardiovascular response to awakening, even though they did correlate with the cortisol response to awakening. These laboratory IBI data provided seemingly reliable measures of trait-like HR and HRV as correlations between sessions on the same day and between days were consistently high. Furthermore, relationships between the

CAR and HR/HRV estimates from laboratory recordings were consistent with those found from the peri-awakening recordings, i.e. steady state measures from the pre- and post-awakening periods. This was not surprising as the sedentary laboratory measures of HR/HRV were highly correlated with these pre- and post-awakening measures, reinforcing the conclusion that they represented a trait-like characteristic. These data indicate that within this young and healthy population between-participant differences in the CAR, averaged across two days, were related to trait-like aspects of these indexes of cardiac regulation. These results are currently difficult to interpret but may indicate some shared variance between resting cardiovascular measures and activation of the HPA axis, which is known to substantially contribute to the CAR. More work exploring the relationship between the CAR and measures of CV function are warranted. In particular, it would be interesting to investigate whether these relationships are similar in older populations as well as those with known cardiovascular pathology.

It is interesting that the CAR was found to be inversely related to trait SDNN, LF and HF HRV whilst no significant associations with the LF/HF ratio were seen. This suggests that an attenuated CAR was associated with increased overall variability in heart IBIs, assumed to result from raised sympathetic and parasympathetic modulation of the heart beat, rather than dominance of either the sympathetic or parasympathetic branch of the ANS. This is an intriguing finding which is difficult to interpret. Previous research examining marker qualities of HRV measures with regard to variables in the psychosocial domain has mostly focused on altered activity of the specific ANS branches or their respective balance, e.g. enhanced parasympathetically mediated HRV has been hypothesised to be associated with an improved capacity to regulate emotions ([Porges, 1997, 2001](#); [Thayer and Lane, 2000, 2009](#)). Indexes of overall HRV or total power, such as SDNN, have mostly been related to overall cardiac health; e.g. depressed overall HRV after myocardial infarction serves as a predictor of mortality and arrhythmic complications ([Task Force, 1996](#)).

In the current study no significant associations between measures of either the CAR or HRV and self-report measures of difficulties in emotion regulation or chronic stress were found which makes it difficult to interpret the findings in terms of psychophysiological 'well-being'. It remains possible that the observed trait-like relationship between the CAR and overall autonomic activity might be the result of either a currently unknown physiological link or of shared relationships with a third variable not captured in the present study. However, based on the current results no prediction regarding which of these possibilities is more likely can be made.

A potential limitation of the current study is that the timing of saliva sampling was not objectively verified, e.g. via electronic monitoring containers ([Kudielka et al., 2003](#)). Similarly, the fact that no information on menstrual stage and use of oral contraceptives (OC) in female participants was obtained should be considered as a possible limitation; however, since previous research has revealed no or only relatively minor influences of these factors on the CAR (see [Fries et al., 2009](#)) and no sex differences in the CAR or measures of HRV were observed in the current study (data not shown), it is unlikely that this has strongly influenced the present results. In addition, it is possible that aspects of the

post-awakening protocol might have influenced the current findings. More specifically, participants were instructed to sit up on awakening and to remain seated for the first 15 min post-awakening to obtain a more controlled IBI recording. Subsequently, they were allowed to move around freely. Whilst we did not find a significant influence of motility levels on HR/HRV measures in the present study, sitting up from a supine position as well as standing up and moving around clearly stimulate cardiac activity in addition to the process of awakening (Huikuri et al., 1994). Our decision to refrain from instructing participants to stay in a supine position for the sampling period was based on the notion that this would reduce burden on participants and would reduce the risk of participants falling asleep again, which is important for the validity of CAR assessment. However, replication of the current study with participants remaining a supine position in a sleep laboratory, where the state of wakefulness can be monitored, would certainly allow a more clear determination of pure awakening-induced input.

In conclusion, the current study has provided evidence for a trait-like association between the CAR and HR as well as overall HRV such that those participants with higher HR and lower SDNN, LF and HF HRV manifest with a reduced dynamic increase in cortisol secretion following awakening. These associations were not mediated by the psychosocial variables of emotion dysregulation and stress. The failure of the current study to show a relationship between the CAR and concurrent awakening-induced changes in HR/HRV point to the complexity of the regulation of the CAR which is thought to be initiated by activation of the HPA axis as well as by direct neural stimulation to the adrenal cortex.

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Conflict of interest

There are no conflicts of interest.

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