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Gartland, N, O'Connor, DB, Lawton, R et al. (1 more author) (2014) Exploring day-to-day dynamics of daily stressor appraisals, physical symptoms and the cortisol awakening response. Psychoneuroendocrinology, 50. pp. 130-138. ISSN 0306-4530

https://doi.org/10.1016/j.psyneuen.2014.08.006

Exploring day-to-day dynamics of daily stressor appraisals, physical symptoms and the cortisol awakening response.

Running title: Day-to-day dynamics of the cortisol awakening response

Abstract

Stress is associated with the secretion of cortisol throughout the day, but less is known about the dynamic effects of stress on the cortisol awakening response (CAR). More widely, knowledge of the causal factors and functions of the CAR are also not fully understood. This study explored: (1) the effects of daily stressors on the next day CAR and; (2) the effects of the CAR on same day physical and affective outcomes. Sixty-four participants completed a daily diary, reporting on the occurrence of daily stressors and stress appraisals, physical symptoms, and affect. Cortisol was measured at 0, 15, 30, and 45 minutes after awakening to provide measures of the CAR on 3 consecutive work days. Stress appraisal was found to negatively predict the CAR, such that where stressors were appraised as more stressful (where perceived demands exceeded resources), the CAR increased less the following morning. Furthermore, the CAR significantly predicted same-day physical symptoms such that a lower CAR was associated with more physical symptoms. This study provides evidence for a pathway through which daily stressors may influence physical wellbeing, and highlights the importance of appraisals for future stress-based cortisol research.

Keywords: cortisol awakening response; daily stress; stress appraisal; physical symptoms; multi-level modelling.

Introduction

The diurnal pattern of cortisol secretion is characterised by two distinct phases: the cortisol awakening response (CAR), a steep rise in cortisol which occurs in the first 45 minutes after waking, and diminishing levels of cortisol through the rest of the day (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010; Fries, Dettenborn, & Kirschbaum, 2009; Pruessner et al., 1997). The CAR has been a popular topic of recent research, though its function and regulation are not yet fully understood. However, there is growing evidence to suggest that cortisol levels are linked to both stress and health (Bellingrath, Weigl, & Kudielka, 2008; Miller, Chen, & Zhou, 2007; O'Connor, Walker, Hendrickx, Talbot, & Schaefer, 2013; Wirtz et al., 2007), and therefore the dynamics of cortisol secretion could represent important physiological mechanisms involved in the negative impact of stress on health.

Much of the research into the relationships between cortisol, stress and health has focussed on cross-sectional or aggregated data, collected over multiple days. However, recent research has observed that intraindividual day-to-day variations in the CAR may be associated with daily experiences and state-specific factors (Dahlgren, Kecklund, Theorell, & Akerstedt, 2009; Hellhammer et al., 2007; for review see Law, Hucklebridge, Thorn, Evans, & Clow, 2013; O'Connor et al., 2013; Stalder, Hucklebridge, Evans, & Clow, 2009; Thorn, Hucklebridge, Evans, & Clow, 2009). Research has also demonstrated the value of analysing this variability with multi-level modelling (Almeida, Piazza, & Stawski, 2009; van Eck, Berkhof, Nicolson, & Sulon, 1996). Crucially, examining the CAR repeatedly within a single participant controls for stable trait characteristics and therefore allows for investigations into state factors. A variety of daily experiential factors have been shown to be related to ambulatory cortisol levels, including daily stress events, affect/mood, and anticipation of obligations (Jacobs et al., 2007; for review see Kudielka, Gierens, Hellhammer, Wust, &

Schlotz, 2012; Stalder, Evans, Hucklebridge, & Clow, 2010a; van Eck et al., 1996). In a 28-day study of daily experience and cortisol, low cortisol levels in the morning were related to anxiety, exhaustion, sleepiness at awakening and poor health the day before; while high levels of cortisol in the evening were related to stress and poor health (Dahlgren et al., 2009). Doane and Adam (2010) demonstrated the importance of day-to-day designs by showing that prior-day feelings of loneliness, worry or stress produced disruptions to the CAR the following morning, but not when the CAR variables were averaged across the study days. In addition, loneliness and stress had divergent effects on the CAR (prior-day loneliness predicted increases in the CAR the following morning, and prior-day stress predicted decreases in waking cortisol levels the following morning), highlighting the differential effects of different forms of emotional strain.

In the study of day-to-day stress, hassles have often been measured simply through frequency or intensity. However, recent research has highlighted the importance of daily hassle appraisals and demonstrated the increased predictive utility of appraisals as compared to measures of frequency and intensity (Gartland, O'Connor, & Lawton, 2012; Gartland, O'Connor, Lawton, & Ferguson, 2014). Appraisals are the interpretations of events in terms of their benefit or harm for the individual; the transactional model of stress posits two dimensions: primary and secondary appraisals (Lazarus & Folkman, 1984). Primary appraisal evaluates the risks or demands of the situation (i.e., high versus low), while secondary appraisal evaluates the availability of resources and whether anything can be done to alter the outcome. In previous research, a ratio of primary to secondary appraisal has been calculated, which reflects the extent to which these appraisals match one another (Gartland et al., 2014; Schneider, 2008; Tomaka, Blascovich, Kibler, & Ernst, 1997), and is consistent with the theory of primary and secondary appraisal interplay (Lazarus & Folkman, 1984). Crucially, this ratio provides a way of looking at appraisals which accounts for the specific interaction

between one's demands and resources at the point of a single stressor, based on the premise that it is only when demands outweigh resources that a hassle will be experienced as stressful.

Interestingly, research has begun to emerge that suggests cortisol levels are related to the same-day reporting of fatigue and physical symptoms (Adam, Hawkley, Kudielka, & Cacioppo, 2006). Specifically, lower levels of cortisol at waking were associated with greater fatigue and physical symptoms during the rest of the day. While cross-sectional studies have demonstrated associations between low basal cortisol levels and fatigue-related conditions (e.g. Pruessner, Hellhammer, & Kirschbaum, 1999), the day-to-day design of Adam et al.'s study allowed for the simultaneous modelling of prior- and same-day fatigue and physical symptoms. This analysis showed that prior-day symptoms were not related to waking levels of cortisol, but same-day symptoms were; therefore, the likely causal direction of this effect was from low cortisol levels to greater fatigue and physical symptoms. As Adam et al. point out, it remains to be seen whether this effect extends to clinical levels of fatigue or symptoms, though some support has been found in clinical trials with chronic fatigue patients, where the administration of glucocorticoids produced short-term alleviation of fatigue symptoms (Cleare et al., 1999; McKenzie et al., 1998). The effect of waking cortisol on subsequent physical symptoms highlights the significance of cortisol for health outcomes at the daily level. The current study was designed to test this prospective effect of cortisol on same-day physical symptoms, but also on same-day positive and negative affect. Affect is a measure of emotional well-being, and is also related to a variety of health measures, including immune-function, hypertension and mortality (Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002; Pressman & Cohen, 2005; Steptoe, Dockray, & Wardle, 2009; Wilson, Bienias, de Leon, Evans, & Bennett, 2003).

The current research aimed to add to this growing body of literature by i) investigating the effects of daily stressor appraisals on day-to-day levels of cortisol across a

4-day period, ii) to test whether the CAR predicted same-day physical symptoms and affect during the same time window, and iii) to investigate the between-subject associations of the CAR with daily stressor appraisals and physical symptoms.

Methods

Design and Participants

Sixty-four participants (mean age of 29 years, range 20-49, 87.5% Caucasian, 42 females) were recruited from staff and graduate students at a University in the North of England via posters, email, and in person. Participants were included in the study if they met the following criteria: (1) aged between 18 and 50 years, (2) non-smoker, (3) had not been to see a psychologist/psychiatrist in the last 6 months, (4) did not have a hormonal disorder such as polycystic ovary syndrome, (5) did not take steroid-based medication, (6) did not have any significant current or past medical history such as diabetes and (7) female participants had to be pre-menopausal. While taking the contraceptive pill was not an exclusion criterion, participants were asked to report this information at baseline. Twenty-one of the 42 female participants reported taking the contraceptive pill. Approval from the University ethics committee was established before commencement of data collection. This study employed an interval contingent daily diary design and participants received a £15 honorarium upon completion of this study. Participants completed a baseline questionnaire, a daily diary, and collected saliva samples to measure the CAR on 3 consecutive days.

Procedure

Participants were first asked to complete an on-line demographics questionnaire. Participants who met the inclusion criteria were then invited to attend an individual face-to-face laboratory session with the primary researcher, in which the study protocol was explained and participants were provided with the daily diary and salivettes. Participants returned these materials after completing 3 days of saliva sampling.

Daily Diary

The daily diary was completed at the end of the day, starting the evening before the initiation of saliva sampling. The diary consisted of several measures:

- Daily Hassles. A record for all the daily hassles experienced during the day. Daily hassles were defined as "events, thoughts or situations which, when they occur, produce negative feelings such as annoyance, irritation, worry or frustration, and/or make you subjectively aware that your goals and plans will be more difficult or impossible to achieve as a result" (O'Connor, Jones, Conner, McMillan, & Ferguson, 2008, p. S20). Participants were requested, using free responses, to report each stressor or hassle experienced and then to rate its intensity on a scale extending from 'Not at all Intense' (1) to 'Very Intense' (5). The time of occurrence for each hassle was also reported. A total of 538 hassles were reported by participants, with an average of 2.1 hassles reported each day.
- Modified SAS (Gartland et al., 2012). This is an 8-item scale with 5 primary appraisal items which measure the demands of the situation (e.g. 'How threatening did you find the daily hassle to be?'; Cronbach's $\alpha = .94$) and 3 secondary appraisal items which measure the availability of resources (e.g. 'Before the hassle was resolved, how well did you think you could manage the demands imposed on you by the daily hassle?'; Cronbach's $\alpha = .94$). The appraisal of each individual hassle was rated on a scale from 1 (Not at all) to 8 (To a very large extent) and the mean score for each scale calculated. The appraisal ratio was calculated by dividing the primary appraisal by the secondary appraisal and a high score (i.e., high ratio) is indicative of where perceived demands outweigh perceived resources.
- The PANAS (Mackinnon et al., 1999). The shortened PANAS was used; this is a 10-item measure of daily affect, which includes 5 positive affect items (e.g. excited, alert; Cronbach's $\alpha = 0.81$) and 5 negative affect items (e.g. nervous, distressed; Cronbach's $\alpha = 0.78$). Participants were asked to respond with regard to the whole day. The positive and negative items were averaged to give daily positive and negative affect scores.

- Physical Symptom (PS) Reporting (Ferguson, Cassaday, Erskind, & Delahaye, 2004). A 12-item measure of physical symptom experience was completed daily. This scale asks participants to what extent they have experienced a range of physical symptoms during the day (i.e. headache, breathlessness, irregular bowel movements). Responses were made on a scale from 1 (did not experience the symptom) to 6 (experienced the symptom very severely). As described by Ferguson, both a frequency score and a severity score was calculated (Ferguson, 2008). Frequency was a dichotomised score, calculated by counting the number of symptoms for which a rating of greater than 1 was given. Severity was calculated by summing the total scale score, as this gave an indication of the extent to which any symptoms had been experienced.

Cortisol measurement

Participants were asked to take their own saliva samples over 3 working days, at waking (0 minutes; taken while the participant was still in bed), 15 minutes post-awakening, 30 minutes post-awakening, and 45 minutes post-awakening. Cortisol was collected from saliva, using Salivettes (Sarstedt, Germany); participants collected a sample by chewing on the swab for 1-2 minutes, and then storing it in a polypropylene tube provided. The importance of the sample times was impressed on participants: it was made clear that it would be apparent from the cortisol levels if they had failed to adhere to the protocol through both discussion during the laboratory session as well as being highlighted on the cortisol collection instruction sheet. Participants were asked to report the actual time of sampling if it differed from the instructed time of sampling. Participants were instructed to refrain from eating, drinking (except water), smoking, and tooth brushing until after the 45 minute post-awakening sample had been taken. Participants were given the option to have reminder text messages sent to their phone for the 3 nights prior to the saliva sampling days, in order to

help maximise compliance to the cortisol sampling protocol (49 participants received text prompts).

Cortisol samples were returned to the researcher the day after the final cortisol sample was taken; once returned, the samples were stored at -20°C or lower until assay. Cortisol levels were determined by using a competitive enzyme-linked immunosorbent assay kit (ELISA) designed for analysing saliva. Intra-assay and inter-assay coefficients of variation of this assay are 5.86% and 6.29%, respectively. The waking level of cortisol (S1) and the area under the curve with respect to increase (AUCi) were calculated as measures of the CAR, as described in relevant literature (Clow et al., 2010; Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

Data Screening

The cortisol data were screened for outliers. Outliers were defined as any value greater or lesser than 3 standard deviations around the mean. Nine outliers were identified; 8 outliers came from two participants whose data was removed as they provided insufficient data due to outliers and suspected non-compliance (below). The single remaining outlier was capped at 2 standard deviations above the mean. In order to correct for significant positive skew in the cortisol data, the raw cortisol values were log transformed.

Strict adherence to timing protocol is required in order to accurately measure the CAR because of its dynamic nature. Research has demonstrated that if the ‘waking’ cortisol sample is taken after waking (as measured by self-report or electronically tagged containers), then the CAR is reduced (Kudielka, Broderick, & Kirschbaum, 2003; Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004). This is suggested to be because the CAR has already commenced, and the measurement is taken when cortisol levels have already increased. Thorn, Hucklebridge, Evans and Clow (2006) reported that a healthy individual who does not show any increase in cortisol between waking and 30 minutes post-awakening can be suspected

non-compliant (SNC). This is based on electrocardiography (ECG) and movement evidence which shows that on average, people demonstrating no rise in this time period woke up 42 minutes before their first sample (Kupper et al., 2005). Therefore, the current data were screened for days when there was no rise in cortisol after the first sample. Data were also screened for reported non-compliance (RNC), where participants reported taking their CAR samples more than 10 minutes later than required by the protocol. On the basis of these criteria, 4 participants were removed from the CAR analysis completely as less than 1 full day of accurate data was provided (due to RNC, SNC, and cortisol outliers). In addition, there were 3 further instances of RNC and 24 further instances of SNC, leaving 153 days of data across 60 participants out of the potential 177 days. Two individual cortisol values were missing in the CAR data, therefore, these were imputed using the mean for that sample time of the other sample days for the same participant.

Analytic strategy

The data were analysed utilising Multi-level Modelling using HLM7 (Raudenbush, Bryk, Cheong, Congdon, & du Toit, 2011). The data contained a 2-level hierarchical structure; Level 1 representing within-person variation (e.g. daily variation in cortisol, number of hassles), and Level 2 representing between-person variability (e.g. age, gender). Level 1 predictors were group mean centred, while the Level 2 predictors were grand mean centred. In order to test the effects of stress on the CAR, the three stress measures (total number of hassles, average hassle intensity and the appraisal ratio) were entered into separate models in order to identify their individual effects. Note that in the case of frequency of physical symptoms experienced, as it was a count variable, it was modelled as a Poisson (Raudenbush, Bryk, Cheong, & Congdon, 2004).

In preliminary analyses, control variables were entered at Level 2 (age, gender, BMI); however, no effects of these on outcome variables were found and the inclusion of these

variables did not change the subsequent results. Therefore, in order to present the most parsimonious models only the Level 1 models are presented. Please note that the person-specific variance was still modelled in these analyses. Furthermore, in previous research, time of waking has been controlled for in models of the CAR (Dahlgren et al., 2009; Stalder et al., 2009). In the current data set, no significant correlations were found between waking time and either the S1 or the CAR AUCi ($r = -.24$, $p = .77$; $r = .04$, $p = .62$ respectively); therefore, waking time was not included in these analyses (average waking time was 7:28am, ranging from 5:40am to 11:00am). It is also important to note that stress did not predict the next day waking time, therefore, ruling out the possibility that any observed effects of stress on the CAR are accounted for by stress yesterday causing participants to awake earlier (or later) the following day.

Three models were used in the analysis, and their general forms are expressed by the following equations:

Model 1 was designed to test the main effects of hassle variables on the next-day CAR:

$$\text{CAR} = \beta_{00} + \beta_{10}(\text{Hassle Variable}) + \varepsilon$$

where β_{00} indicates the mean level of the CAR; β_{10} indicates the average size of the relationship between the hassle variable and the CAR, and ε the error term.

Model 2 was based on that of Adam et al. (2006), who entered prior- and same-day physical symptoms simultaneously into a model predicting morning cortisol, in order to determine which variable was more strongly associated with the CAR. Here, physical symptoms and daily affect were entered separately into models predicting the CAR:

$$\text{Cortisol} = \beta_{00} + \beta_{10}(\text{Same-Day Health Variable}) + \beta_{20}(\text{Prior-Day Health Variable}) + \varepsilon$$

where β_{00} indicates the mean level of cortisol; β_{10} indicates the average size of the relationship between the Same-Day Health Variable and cortisol; β_{20} indicates the average size of the relationship between the Prior-Day Health Variable and cortisol, and ε the error term.

As Model 2 did not test the predictive relationship between the CAR and same-day health outcomes, Model 3 was designed to test the causal direction of any associations identified by Model 2. Model 3 investigated the effect of the CAR on same-day health outcomes while controlling for prior-day health outcomes:

Same-Day Health Outcome = $\beta_{00} + \beta_{10}$(CAR) + β_{20} (Prior-Day Health Outcome) + ε

where β_{00} indicates the mean level of Same-Day Health Outcome; β_{10} indicates the average size of the relationship between the CAR and Same-Day Health Outcome; β_{20} indicates the average size of the relationship between the Prior-Day Health Outcome and Same-Day Health Outcome, and ε the error term.

Finally, a between-subjects analysis was carried out. Subject-level appraisal, physical symptom and cortisol values were calculated by averaging the data across all study days and Pearson's correlations were then calculated.

Results

Descriptive statistics

Descriptive statistics were calculated for all main Level 1 and Level 2 variables (Table 1). The scores presented in Table 1 are in line with those expected from healthy volunteers; the average BMI is within the normal range of 18.5 to 25, participants reported their general health on average to be between ‘excellent’ and ‘good’, and low scores were reported for major prior health issues and the general experience of physical symptoms.

Table 1 about here.

Testing the relationships between daily hassles/appraisals and the next-day CAR

The analysis of Model 1 demonstrated that the appraisal ratio was found to negatively predict the CAR AUCi, such that where hassles were appraised as more stressful (where perceived demands exceeded perceived resources) the CAR increased less the following morning (Table 3). Neither total number of hassles nor average hassle intensity were found to influence the next-day CAR, and no effects of the stress measures were found on the S1.

Table 3 about here.

Testing the direct effects of the CAR on same-day health outcomes

The analysis of Model 2 demonstrated that only the same-day physical symptoms had a significant association with the CAR AUCi, when same-day and prior-day physical symptoms were simultaneously entered (Table 4). This was a negative relationship such that lesser increases in the CAR in the morning were associated with the experience of greater physical symptoms throughout the same day, and was significant for both symptom severity and frequency. No associations between the CAR and daily affect were found, and no effects were identified in relation to S1.

The association between the CAR AUCi and same-day physical symptoms was then further explored using Model 3. This analysis demonstrated that the CAR AUCi significantly predicted both same-day physical symptom severity and frequency (see Table 5). The relationship was significant even after controlling for the relationship between prior- and same-day physical symptoms. The nature of this relationship was such that having a lower increase in the CAR in the morning predicted the reporting of more frequent and severe physical symptoms at the end of the same day.

Testing between-subject associations between the CAR, appraisals, physical symptoms and affect

Between-subject Pearson's correlation analyses were run to test for associations of the CAR with hassle appraisals, physical symptom severity and frequency, and/or daily affect. No significant associations were found (data not shown).

Discussion

This study investigated the relationships between daily experiences and the CAR. The appraisal ratio of daily hassles was found to predict the CAR the following morning, such that mismatched appraisals (where perceived demands outweighed perceived resources) led to a lower CAR increase the following day. Evidence was also found to suggest that the CAR predicted the experience of physical symptoms later in the day, where having a lower CAR was associated with more frequent and severe physical symptoms throughout the rest of the day. The CAR was not associated with levels of affect later in the day. These findings provide further insights into the functionality of the CAR and widen our knowledge of what day-to-day factors influence cortisol levels.

The current study is the first to assess the effects of appraisals of daily hassles on the next-day CAR. Appraisals of greater stress were associated with a lesser increase in cortisol the following day. It is important to note that the number and intensity of hassles did not predict the next-day CAR; this suggests that appraisals of hassles are critical and are capable of explaining more variance than general measures of frequency and intensity. Research into appraisals of acute stress has focussed on immediate cortisol responses rather than the CAR. Gaab et al. (2005) studied the cortisol response to the TSST, and found that reporting more threatening/challenging appraisals was correlated with an increased cortisol response to the TSST. Day-to-day studies looking into the associations between stress related variables and the CAR have provided evidence for a positive association between anticipated obligation or tension and the CAR, but have not found relationships between prior-day obligations or tension and the CAR or measured the relationships between prior-day stress and the CAR (Stalder et al., 2010a; Stalder, Evans, Hucklebridge, & Clow, 2010b). However, a number of studies have shown that high levels of trait stress are associated with lower cortisol levels in the morning (e.g. O'Connor et al., 2009; Thorn et al., 2006). Therefore, the literature shows a

number of different effects on the CAR relating the various measures of stress. Nevertheless, the current study is the first investigation of the effects of appraisals of specific daily hassles on the next-day CAR. It is possible that the after-effects of daily hassles may be distinct from the effects of more generalised or anticipated stress, and may have different effects on hormone regulation. Importantly, the day-to-day analysis of this data suggests that rather than a pervading reduction in the CAR over time, the lower CAR was seen only after days when hassles were appraised as stressful (demands outweighed resources). In another day-to-day study, general stress was shown to predict higher levels of cortisol at bedtime while anxiety was shown to predict lower levels of cortisol the next morning (Dahlgren et al., 2009). Therefore, the finding that the appraisal ratio predicted lower increases in cortisol the next day is broadly supportive of this work. It is also important to note that in the current study stress was not related to the next day waking time, thus any observed effect cannot be accounted for by a delay in waking. A possible suggestion to explain these findings is that daily stress leads to increases in same-day cortisol levels, followed by lower secretion the following morning. Unfortunately, the design of our study could not test this possibility as cortisol samples were not linked to hassles, but it does pose an interesting question for further research.

The current study also found that a lower increase in the CAR in the morning predicted the experience of more physical symptoms throughout the day ahead. This supports an effect identified in previous research (Adam et al., 2006), however, Adam et al. demonstrated an effect of the S1 on same-day physical symptoms rather than the CAR AUCi. Nevertheless, these findings indicate that the CAR may not just be a marker of ill-health, but changes in cortisol secretion may have a direct influence on the experience of symptoms. These findings have important implications, as they provide some insight into the functionality of the CAR, which is yet to be fully understood. Interestingly, the most

frequently reported physical symptom in the current study was feeling ‘weak/fatigued’, thus suggesting that exhibiting a lower CAR in the morning may have a specific effect on feelings of fatigue later in the same day. Further evidence in support of this idea comes from a longitudinal study of cortisol and fatigue over a 5 year period, which demonstrated that low waking cortisol and a flattened cortisol slope through the day were associated with an increased risk of future fatigue (Kumari et al., 2009). It is interesting to note that previous studies have found an association of waking cortisol levels with future fatigue and physical symptoms. In the current study, only the increase in the CAR was associated with physical symptoms at the end of the same day. These two aspects of the CAR have been suggested to reflect distinct physiological processes (Clow et al., 2010). Waking cortisol levels may reflect the reduction of adrenal sensitivity to ACTH by the suprachiasmatic nucleus extra-pituitary pathway (pre-awakening), while the increase in the CAR may reflect the activation of the HPA axis and increased adrenal sensitivity to ACTH (post-awakening). Therefore, both of these systems (and their interactions) may be implicated in the increase of fatigue. The CAR has also shown associations with sleep quality, thus, it is possible that poorer sleep quality impacts both the CAR and subsequent feelings of fatigue (Lasikiewicz, Hendrickx, Talbot, & Dye, 2008; Waye, Clow, Edwards, Hucklebridge, & Rylander, 2003). Further research will be required to identify the effects of each unique aspect of the CAR and the physiological mechanisms underlying these associations.

Taking these two findings together, this study demonstrates that more stressful appraisals are associated with lower increases in the next-day CAR, but also that lower increases in the next-day CAR predict the experience of a greater number of more severe same-day physical symptoms. This suggests that stressful hassles might lead to a lower CAR increase and thus, perhaps, more physical symptoms on the day after the hassles were experienced. This provides preliminary evidence for a pathway through which daily hassles

may influence health, and highlights the importance of appraisals for future stress-based cortisol research. Further research is needed in order to test this hypothesised mediation pathway specifically.

All the findings of the current study relate only to the increase in cortisol levels of the CAR (AUCi) and not to waking levels of cortisol (S1). As described above, the S1 and the CAR AUCi may represent markers of two distinct physiological mechanisms. Therefore, in relation to the appraisal finding, it may be that the AUCi is more sensitive to changes in stress-related variables as it is a marker of HPA axis activation. Again, research is needed to investigate the representation of stress responses through the dynamics of the CAR.

Finally, the between-subject analysis demonstrated that no associations between the study variables existed when examined at the subject level. This suggests that it is not the case that averaged hassle appraisals or averaged physical symptoms are generally associated with lower CAR increases; rather, it is evidence that the changes in the CAR from day-to-day are associated with changes in daily experience. These relationships can be said to be at the state level rather than the trait level. This adds to the growing opinion that measuring and analysing day-to-day changes in experience is important for gaining the full picture of what influences the CAR and what effects the CAR may have on daily experience (for review see Law et al., 2013).

A number of limitations of the current study ought to be briefly acknowledged. We recognise that many studies discuss and address the need for objective tests of participant adherence to the cortisol sampling protocol, typically using electronic containers for Salivettes which record the time at which they were opened (for review see Clow, Thorn, Evans, & Hucklebridge, 2004). Research has argued that healthy non-compliers can be identified through their cortisol profiles, as those showing no increase were more likely to have taken their first saliva sample too late, thus measuring from mid-way through the CAR

(Thorn et al., 2006). Therefore, the methods used in the current study have been shown to be appropriate for healthy participants, for whom there should be a rise in cortisol levels in the morning (e.g. O'Connor et al., 2009; Thorn et al., 2006). Moreover, we included a number of methodological features to the participant study briefings that are likely to have substantially reduced protocol adherence problems (e.g., explaining that the experimenters could identify protocol non-adherence in the samples, ensuring that participants kept diaries and received reminders). Nevertheless, further research is required which objectively tests protocol adherence within this context.

In conclusion, this study is novel in its investigation of the effects of daily hassles and hassle appraisals on the next-day CAR and the consequences of the CAR for same-day physical symptoms. The current study also supports previous research which demonstrates that the CAR is predictive of same-day physical symptoms; crucially implicating the CAR in daily physical functioning and potentially in the aetiology of ill-health.

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Table 1. Descriptive statistics for Level 2 (person) and Level 1 (daily) variables (cortisol values are un-transformed).

	Mean (Range)	Std. Deviation
LEVEL 2		
Age	28.97	6.95
Height (in)	67.06	4.34
Weight (lbs)	150.16	30.68
BMI	23.38	3.46
Self-rated general health	1.78 (1-4)	0.63
PS severity	19.58 (12-72)	6.22
PS frequency	3.88 (0-12)	2.50
Positive Affect	3.56 (1-5)	0.67
Negative Affect	1.89 (1-5)	0.59
LEVEL 1		
Hassle Variables:		
Total number of hassles	2.11	1.35
Average hassle intensity	2.71 (0-5)	1.24
Average primary appraisal for all hassles	3.09 (0-7)	1.65
Average secondary appraisal for all hassles	4.66 (0-7)	1.91
Average appraisal ratio for all hassles	.80 (0-7)	0.55
Affect and Physical Symptom Variables:		
Positive affect	2.76 (1-5)	0.82
Negative affect	1.56 (1-5)	0.65
PS severity	16.99 (12-72)	5.82
PS frequency	2.39 (0-12)	2.23
Cortisol:		
Waking level (S1)	7.93	3.80

15 minutes post-awakening	10.48	3.95
30 minutes post-awakening	11.82	4.39
45 minutes post-awakening	10.94	3.56
CAR AUCi	119.06	106.39

BMI body mass index, PS physical symptoms, CAR AUCi cortisol awakening response with respect to increase.

Table 2. Frequency table for the daily experience of physical symptoms across all days and all participants.

Physical Symptom	Did not experience	Experienced very mildly	Experienced mildly	Experienced Moderately	Experienced Severely	Experienced very severely
Headache	168	29	27	13	10	6
Dizziness	222	17	7	3	2	2
Palpitations	221	17	4	8	2	1
Weak/Fatigued	128	47	32	23	14	8
Upset Stomach	201	23	18	4	3	4
Pain in Limbs	192	30	12	10	5	3
Pain in Joints	180	20	21	17	9	5
Sexual Problems	249	2	1	0	0	0
Breathlessness	222	13	7	9	1	1
Sweatiness	186	36	14	13	3	1
Tingling in Hands and Feet	236	9	5	3	0	0
Irregular Bowel Movements	220	13	13	4	2	2

Table 3. Level 1 effects of hassle number/hassle intensity/hassle appraisal ratio predicting the next-day CAR AUCi.

HLM effect	Symbol	Coeff	SE	p
CAR AUCi				
Intercept	β_{00}	5.99	0.60	<.001
Level-1 slope				
Total Hassles - CAR AUCi	β_{10}	0.15	0.46	0.74
CAR AUCi				
Intercept	β_{00}	5.99	0.60	<.001
Level-1 slope				
Avg Hassle Intensity - CAR AUCi	β_{10}	-0.01	0.15	0.96
CAR AUCi				
Intercept	β_{00}	5.94	0.61	<.001
Level-1 slope				
Appraisal Ratio - CAR AUCi	β_{10}	-2.76	1.16	0.02

CAR AUCi cortisol awakening response with respect to increase.

Table 4. Level 1 effects of same-day and prior-day physical symptoms and affect predicting the CAR AUCi.

HLM effect	Symbol	Coeff	SE	p
PS Severity - CAR AUCi				
Intercept	β_{00}	5.98	0.60	<.001
Level-1 slopes				
Same-day PS severity - CAR AUCi	β_{10}	-0.38	0.16	0.02
Prior-day PS severity - CAR AUCi	β_{20}	0.03	0.18	0.87
PS Frequency - CAR AUCi				
Intercept	β_{00}	5.98	0.60	<.001
Level-1 slopes				
Same-day PS frequency - CAR AUCi	β_{10}	-0.83	0.40	0.04
Prior-day PS frequency - CAR AUCi	β_{20}	0.03	0.41	0.94
Positive Affect – CAR AUCi				
Intercept	β_{00}	5.99	0.60	<.001
Level-1 slopes				
Same-day Positive Affect - CAR AUCi	β_{10}	0.59	0.87	0.50
Prior-day Positive Affect- CAR AUCi	β_{20}	0.45	0.89	0.62
Negative Affect – CAR AUCi				
Intercept	β_{00}	5.98	0.60	<.001
Level-1 slopes				
Same-day Negative Affect - CAR AUCi	β_{10}	-0.56	0.996	0.58
Prior-day Negative Affect - CAR AUCi	β_{20}	-0.10	0.998	0.92

PS physical symptoms, CAR AUCi cortisol awakening response with respect to increase.

Table 5. Level 1 effects of CAR AUCi predicting same-day physical symptoms severity and frequency, controlling for prior-day physical symptoms.

HLM effect	Symbol	Coeff	SE	p
CAR AUCi - Severity				
Intercept	β_{00}	16.77	0.67	<.001
Level-1 slopes				
CAR AUCi – Same-day PS	β_{10}	-0.23	0.07	0.003
Prior-day PS severity – Same-day PS	β_{20}	-0.20	0.12	0.11
CAR AUCi - Frequency				
Intercept	β_{00}	2.31	0.25	<.001
Level-1 slopes				
CAR AUCi – Same-day PS	β_{10}	-0.07	0.03	0.03
Prior-day PS freq – Same-day PS	β_{20}	-0.30	0.11	0.01

CAR AUCi cortisol awakening response with respect to increase, PS physical symptoms.