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Associations between psychosocial state variables and the cortisol awakening response in a single case study

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Summary The current study investigated intra-individual associations between psychosocial state variables and the cortisol awakening response (CAR) in a detailed case study of a 27-year-old healthy male (TS) carried out over 50 measurement days, occurring at 3-day intervals. Quantitative diaries capturing psychosocial states were filled out on the evening before each study day as well as 45 min post-awakening on the study day. On each study day, salivary free cortisol was determined at 0, 15, 30, and 45 min post-awakening. Relationships between cortisol measures and psychosocial variables were analysed using correlation analyses and relative predictive input of independent variables was further determined using linear regression analysis. Significant relationships were found between psychosocial state variables and the dynamic of the CAR (area under increase curve; AUC_I). The final regression model for the AUC_I (explaining ~22% of its variability), included an inverse relationship with the level of prior-day happiness and a positive relationship with study-day anticipations of the level of obligations/no leisure. The results are discussed within the context of previous evidence and potential implications for cross-sectional research are highlighted.

1. Introduction

The cortisol awakening response (CAR), the rapid increase in cortisol levels over the first 30—45 min post-awakening, has been found to be associated with a wide range of psychosocial factors and life-style conditions and is increasingly used as a measure in psychobiological research (Fries et al., 2009). However, despite growing evidence suggesting important associations between the CAR and psychosocial functioning, there still remains uncertainty as to the specific nature of such associations as results between studies have frequently been inconsistent (see Clow et al., 2004; Fries et al., 2009). Given this unsatisfactory situation it is important to identify potential reasons for such inconsistencies.

Within this context, an important consideration is that research into the CAR is typically conducted using cross-sectional designs with data accumulated from ~1 to 3 study days per person. While this is a sensible and effective approach to investigate stable, trait-like associations, recent evidence highlights the high amount of state specificity in measures of the CAR (Hellhammer et al., 2007). State variability does not necessarily present a problem for cross-sectional research if variability is random, i.e. factors

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influencing state variability show no systematic associations with experimental groups or variables. If the assumption of randomness of state or situational variability is not tenable, however, findings may well be confounded as unknown state associations might affect results in addition to the trait associations that the study originally set out to examine. A crucial prerequisite to prevent such bias in cross-sectional research is the availability of detailed knowledge about factors associated with intra-individual variability in measures of the CAR.

To date, only a small number of studies have examined associations between changes in psychosocial state variables and the CAR. Adam et al. (2006) examined the influence of both prior-day and same-day experiences on the CAR and found that more self-reported prior-day feelings of loneliness/sadness/threat were associated with a more pronounced dynamic of the CAR the following day (Adam et al., 2006). In research from our own laboratory, day-to-day changes in state arousal at 45 min post-awakening were found to be positively associated with changes in the overall CAR (Thorn et al., 2004) and the dynamic of the CAR (Thorn et al., 2009). In addition, it has been reported that the dynamic of the CAR is elevated on weekdays compared to weekend days (Kunz-Ebrecht et al., 2004; Schlotz et al., 2004; Thorn et al., 2006), even though this was not confirmed by other research (Kudielka and Kern, 2004; De Weerth and Buitelaar, 2005). Findings of weekday/weekend differences were associated with higher levels of perceived stress, and lower control and happiness on weekdays in one study (Kunz-Ebrecht et al., 2004), but were not accounted for by differences in state stress in another study (Thorn et al., 2006). Interpretations of these findings with regards to the function of the CAR have suggested an association with anticipated demands of the day ahead, even though to date this has not been tested directly. While some researchers have seen this more as an adaptive response, providing a 'boost' to meet anticipated demands for the day (Adam et al., 2006), others have related anticipations of negative, stressful events with an elevated CAR (Kunz-Ebrecht et al., 2004; Schlotz et al., 2004).

Recently we corroborated results by Hellhammer et al. (2007) of considerable intra-individual variability in the CAR using a researcher case study over a prolonged period of time (Stalder et al., 2009). In particular we highlighted strong covariation between the first waking cortisol sample and time of awakening, explaining ~38% of its variability. Here we examine intra-individual associations between state psychosocial variables and the CAR from this same researcher case study. Our objective was to use careful research methodology to gain detailed insight into associations between psychosocial variables and the CAR within one person, which can subsequently be tested for their generalisability in larger samples. A particular advantage of the chosen experimental design was that data could be collected over a longer time period than feasible in cross-sectional designs, allowing for considerable within-participant changes to occur. In addition, using a researcher—participant was arguably an efficient way of maximising adherence to protocol. This is crucial as participant non-adherence can be a severe problem in CAR research (Kudielka et al., 2003; Kupper et al., 2005) which has been shown to increase with repeated measurements (Broderick et al., 2004). In order to fully capture potential associations of psychosocial state changes with measures of the CAR, we took a similar approach to Adam et al. (2006) and used quantitative diaries to examine both prior-day and study-day associations. An additional aim was to provide some indication on the importance of the hedonic valence of psychosocial states; hence, we have included measures associated with neutral as well as negative hedonic valence.

2. Method

2.1. Studied case, study design and procedure

As reported in Stalder et al. (2009), the participant (TS) was a 27-year-old healthy, medication free and non-smoking male. He described himself as having a varying sleep pattern, with considerable changes in bed times and awakening times between days, and stated that his occupation as a full-time doctoral student entailed a life-style with no clear weekday—weekend rhythm. He also described himself as a person who wakes up quickly in the morning without 'snoozing'.

The study was carried out on a total of 50 study days, occurring at 3-day intervals. Before going to bed in the evening prior to each study day, the participant filled out an evening diary. Saliva samples were taken immediately on awakening and at 15, 30, and 45 min post-awakening. During this period the participant did not take anything by mouth other than water and did not brush his teeth to avoid abrasion and micro-vascular leakage. After taking the last saliva sample at 45 min post-awakening, the participant filled out the morning diary. The project was approved by the research ethics committee of the University of Westminster and conducted in accordance with the Declaration of Helsinki.

2.2. Materials, cortisol analyses, and psychosocial measures

Pre-labelled Salivettes (saliva sampling devices, Sarstedt Ltd., Leicester, UK) were used for the collection of saliva samples. Cortisol analyses were carried out using a standard enzyme-linked immunosorbent assay protocol (Salimetrics, USA). Simultaneous heart rate and motility readings from an Actiheart monitor (Cambridge Neurotechnology, Cambridge, UK) were used as an objective check on awakening time (as in Kupper et al., 2005).

As the aim of the study was to assess relationships between the CAR and both prior-day and study-day states, as well as study-day anticipations, the same five items were applied with reference to different time periods. In the evening diary, the participant rated the five items retrospectively for the day prior to the study day ('*Feelings about today*'). In the morning diary, the participant rated the same variables as both momentary state measures at 45 min post-awakening ('*Feelings now*') and anticipations about the day ahead ('*Feelings about the coming day*'). Of the five used items, two items were adapted from the Pittsburgh Sleep Diary (PSD; Monk et al., 1994): *Mood* (*very tense* vs. *very calm*; reported in the direction of tension), *Alertness* (*very sleepy* vs. *very alert*). These are assessed via 100 mm visual analogues scales (VAS) and have been validated for use in healthy participants (Monk et al., 1994, 2000). Three more

items were designed to cover further variables of interest: sadness/happiness, obligations and leisure activities. For consistency and ease of repeated use over 50 days the same VAS paradigm as in the PSD items was used. The items were: '*Happiness*' (*very sad* vs. *very happy*), '*Time spent fulfilling obligations*' (*work or other*) (*nothing at all* vs. *very much*) and '*Time spent doing leisure activities*' (*nothing at all* vs. *very much*). In addition, the *Stress Arousal Checklist* (SACL, Mackay et al., 1978) was also completed as part of the morning diary to assess momentary state stress and arousal 45 min after awakening.

2.3. Statistical analyses

Data of two consecutive study days (days 26 and 27) had to be excluded leaving 48 days of valid data for analyses. Exclusion was due to the acute illness (high fever) and medication intake associated with outlying high cortisol values (day 26) and due to insufficient saliva for assaying (day 27). Cortisol data were square root transformed to reduce positive skewness.

A repeated-measures (within-days) ANOVA was performed to examine overall changes in cortisol concentrations following awakening, i.e. the CAR. To examine differences in cortisol values between weekdays and weekend days, this ANOVA was repeated with weekday/weekend as a between-days variable. Significance probabilities for within-days terms in the ANOVAs were corrected for sphericity violation by using the Greenhouse—Geisser method.

For the examination of associations with cortisol data, three measures were used: the level of cortisol on awakening (S1), as well as the areas under the cortisol curve with respect to increase (AUC_I) and ground (AUC_G; as suggested by Pruessner et al., 2003a). Associations between these cortisol measures and psychosocial variables were examined using correlation analyses. As this involved carrying out several tests of statistical significance, adjustments needed to be made in some cases to account for an inflated family-wise error rate. Here a distinction was made between primary variables, for which previous evidence indicated a relationship with measures of the CAR, and secondary variables, which were exploratory in nature and were therefore Bonferroni-corrected. Based on the reviewed evidence primary variables were: (i) the level of sadness/happiness over the day prior to the study day, and on the study day (ii) state arousal and (iii) state stress at 45 min post-awakening, and anticipations of (iv) tension and (v) obligations and leisure activities.

We have previously shown that linear time trends in S1 and AUC_G are present in this data set and that these are largely caused by concurrent trends in awakening time (see Stalder et al., 2009). To prevent confounding influences related to these linear trends, partial correlation analyses, controlling for awakening time, were used to examine relationships between S1 and AUC_G and psychosocial variables (both primary and secondary). As no linear time trends were found for the AUC_I, relationships with psychosocial variables were examined using simple bivariate correlation analyses.

Table 1 Mean values, standard deviations, and ranges of (a) cortisol measures and (b) psychosocial variables for different time points and time periods on the day prior to the study day and the study day.

	Mean	± S.D.	Min	Max
(a) Cortisol (nmol/l)				
Awakening (S1)	6.5	2.2	1.4	11.0
15 min	12.4	4.5	4.9	27.2
30 min	22.1	7.8	9.0	48.4
45 min	19.2	6.6	8.7	34.5
AUC_I	420.0	167.0	86.7	841.3
AUC_G	710.3	188.0	288.1	1248.1
(b) Psychosocial variables				
Prior day:				
Tension	67	19.2	15	92
Alertness	55.5	22	15	92
Happiness	61.8	18.4	13	91
Obligations	46.7	26.6	0	100
Leisure	46.8	27.5	2	98
Study day:				
Tension—45 min	67.4	10.7	36	84
Alertness—45 min	61	16.4	8	85
Happiness—45 min	61.2	7.5	44	80
Arousal (SACL)—45 min	7.7	4.2	0	14
Stress (SACL)—45 min	5	3.8	0	18
Tension—anticipations	64.4	13.9	10	82
Alertness—anticipations	37.7	15.9	8	77
Happiness—anticipations	61.4	9.4	35	89
Obligations—anticipations	47.9	25.8	3	93
Leisure—anticipations	42.7	27	6	94

As a final step, prior-day and same-day psychosocial variables previously found to be significantly correlated with cortisol measures were simultaneously entered into a linear regression model in order to determine their relative predictive input.

3. Results

The participant reported that carrying out the study protocol initially resulted in him waking up earlier than usual (see Stalder et al., 2009, for a detailed discussion). In spite of this, the participant reported a high level of adherence to protocol, with no delays >1 min between the specified and actual saliva sampling times on any of the study days. The accuracy of self-reported awakening times was confirmed by the finding of a mean (±S.D.) difference of 54 s (±71 s) between reported and objectively verified awakening time. Given this close accordance, self-reported data was used in further analyses controlling for awakening time.

Table 1a presents descriptive information for post-awakening cortisol concentrations and composite measures. The ANOVA of changes in cortisol values over the post-awakening period showed a significant within-day effect ($F_{[2,111]} = 140.046$, $p < .0001$), demonstrating the expected cortisol awakening response. This analysis was repeated including weekday/weekend as a between-days factor. The results did not show a significant main effect of weekday/weekend ($F_{[1,46]} = .191$, n.s.) or a sample *x* weekday/weekend interaction ($F_{[2,108]} = .069$, n.s.), suggesting that neither overall post-awakening cortisol levels nor the dynamic of the CAR differed between weekdays and weekend days.

Table 2 Relationships between measures of the CAR and primary (bold) and secondary psychosocial variables.

	Sample 1	AUC_I	AUC_G
Prior day			
Tension	.07	.15	.14
Alertness	.01	.20	.20
Happiness	**−.01**	**−.36***	**−.34***
Obligations/no leisure	−.17	.15	.03
Study day			
State tension—45 min	.05	.15	.16
State alertness—45 min	−.05	.23	.26
State happiness—45 min	.02	.08	.11
State arousal (SACL)—45 min	**−.07**	**.37***	**.33***
State stress (SACL)—45 min	**−.04**	**.20**	**.14**
Tension—anticipations	**−.10**	**.14**	**.02**
Alertness—anticipations	−.20	.16	.10
Happiness—anticipations	−.09	.14	.07
Obligations/no leisure—anticipations	**−.02**	**.36***	**.34***

Results of partial correlation analyses, controlling for awakening time, for Sample 1 and AUC_G and of bivariate correlation analyses for AUC_I; primary variables are indicated in bold writing. Indication of significance for secondary variables is based on Bonferroni-corrected values.

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

Descriptive information of psychosocial state variables for prior-day and study-day time points and periods is shown in Table 1b. Items relating to obligations and leisure activities showed almost perfect relationships with each other (prior day: $r = -.909$, same day: $r = -.895$) and were thus collapsed into a single variable called 'obligations/no leisure' for each time period by computing a single principal component score reflecting their substantial common variance.

Table 2 presents the results of partial and bivariate correlation analyses between cortisol measures and primary and secondary variables. For primary variables, a significant inverse relationship was found between the AUC_I and the reported amount of happiness over the day prior to the study day. Significant positive relationships were also found between the AUC_I and same-day state arousal, as well as anticipations of the factor obligations/no leisure for the study day; these relationships with the AUC_I were also manifest in significant relationships with the AUC_G. However, no significant relationships were found for same-day state stress or anticipated tension over the study day. Also, no significant associations were found between secondary variables and measures of the CAR. There were no significant associations between any of the psychosocial state variables and S1.

Variance in the AUC_G is totally explained by variance in the AUC_I and S1. The fact that no associations were found between any of the state variables and S1 suggested that associations with the AUC_G were largely driven by those with the AUC_I; hence, we only carried out multiple regression analyses for the AUC_I. When the three variables which were significantly correlated with the AUC_I were entered simultaneously into the linear regression model, state arousal lost its statistical significance as a predictor ($\beta = .20$, n.s.), which was likely the case because of a positive association of arousal with the factor obligations/no leisure ($r = .46$, $p < .001$). The final model thus consisted of prior-day happiness ($\beta = -.35$, $p = .01$) and study-day anticipations of the factor obligations/no leisure ($\beta = .35$, $p < .01$; model adjusted $R^2 = .22$).

4. Discussion

In this study we set out to examine the relationship between intra-individual changes in measures of the CAR and psychosocial state variables in an in-depth case study over a protracted period of time. Our results are generally in line with previous evidence and show that a considerable amount (~22%) of the intra-individual variability in the dynamic of the CAR found in the studied case can be explained by taking into account both prior-day levels of sadness/happiness and anticipations about the level of obligations/no leisure for the study day. If confirmed by future research, these findings have important implications regarding potential confounding influences on cross-sectional research.

Based on previous findings, we examined psychosocial state variables over the day prior to a study day. We found an inverse relationship between the amount of prior-day happiness and the dynamic of the CAR, which is in line with results of Adam et al. (2006) who, in a larger sample of older adults examined over 3 consecutive days, found a positive association between their factor lonely/sad/overwhelmed and the dynamic of the CAR. This is a puzzling finding, which

Adam et al. interpret within the context of cross-sectional evidence linking chronic stress (e.g. Schulz et al., 1998; Wüst et al., 2000) or depression (e.g. Pruessner et al., 2003b; Steptoe et al., 2004) with an elevated dynamic of the CAR. A direct causative connection between state stress and the dynamic of the CAR is, however, not suggested for the current case as we did not find an association with prior-day tension. It is tempting to speculate whether part of an adaptive function of the CAR might be to aid acute emotional regulation with regards to the experience of the previous day; e.g., by facilitating a more or less energetic start to the day. Some tentative support for such a view can be seen in the fact that both in the current study as well as the study by Adam et al. significant prior-day associations were not found to carry-over to the study day, but association signs were reversed in both studies.

With regards to study-day associations, we did not find weekday/weekend differences in the dynamic of the CAR, which was expected as the life of the participant over the study period did not follow a clear weekday/weekend pattern in terms of behaviour. However, we found that anticipations of obligations/no leisure, i.e. days anticipated to be busier, were associated with an elevated dynamic of the CAR (AUC_I). This finding corresponds to the view that anticipatory, prospective memory-related processes are of importance for the CAR (e.g. Wilhelm et al., 2007; Fries et al., 2009), a notion that is endorsed by research showing that the CAR is attenuated in patients with hippocampal damage-related memory disorders (Buchanan et al., 2004; Wolf et al., 2005). It has been argued that a potential reason for an association between the CAR and such anticipatory processes could be that the CAR serves an adaptive function providing a 'boost' to meet anticipated demands of the day (Adam et al., 2006). The findings of the current case study are in line with such a view, particularly as anticipation of obligations/no leisure was also found to be positively associated with arousal at 45 min post-awakening, which itself showed a positive association with the dynamic of the CAR.

An alternative view, which for example has been proposed as an explanation for weekday/weekend differences in the CAR, is that anticipations of negative events might work as internal stressors (Schlotz et al., 2004). Our finding that in the studied case the dynamic of the CAR was unrelated to perceived state stress or anticipations of tension give no support to such an interpretation. However, it is important to point out that our results are based on a detailed picture of only a single individual. Individual differences exist in the appraisal of potentially stressful situations (e.g. Schneider, 2008) and it is possible that associations between the CAR and stress or anticipated tension are present in individuals who are more inclined to appraise a busy day as a stressful experience. Unfortunately, none of the previous studies have reported data on anticipations of busyness independent of emotional valence, but have focused on states associated with negative emotional valence (Kunz-Ebrecht et al., 2004; Schlotz et al., 2004; Thorn et al., 2006). Thus, further research will be needed to better understand the exact nature of anticipation valence associated with the dynamic of the CAR.

One motivation underlying this study was to increase available information on intra-individual associations between measures of the CAR and psychosocial variables in order to elucidate potential confounding influences on cross-sectional research. Of the current tentative results, particularly an effect of the anticipated level of obligations/no leisure can be identified as a potential confounding influence on the dynamic increase of the CAR. Published research has frequently compared the CAR profiles of clinical groups with those of healthy controls (e.g. Roberts et al., 2004; Bhagwagar et al., 2005; Gaab et al., 2005; Vedhara et al., 2006), with results of group differences usually being interpreted in terms of the clinical group's pathology. However, the studied groups, despite being matched carefully on demographic grounds, are often examined under very different circumstances, e.g. hospitalised patients vs. home-based controls (Gaab et al., 2005), or largely non-working patients vs. working controls (Roberts et al., 2004). It is likely that these situational differences are also accompanied by other differences, such as prospective memory requirements related to anticipations of the day ahead, i.e. less planning might be required for a hospital day compared to a busy work day. Our preliminary results indicate that such anticipatory processes are associated with the dynamic of the CAR, making potential confounding influences likely. While it is not always feasible to find a perfectly matched control group in terms of situational or psychosocial states, these tentative data indicate that state-related influences pose a potentially powerful confound in studies of the CAR.

In conclusion, our results from a detailed single case study are in line with previous research indicating intra-individual associations between the dynamic of the CAR and psychosocial state variables. Such day-to-day intra-individual variation in the dynamic of the CAR may account for some of the reported inconsistency in associations with trait variables derived from cross-sectional research. Furthermore, the results provide a first tentative indication that study day anticipations of neutral, rather than negative, hedonic valence (related to the general level of busyness but not stress and tension) might be an important determinant of the dynamic of the CAR. Further research using a larger number of participants will be needed to clarify this.

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Conflicts of interest

There are no conflicts of interest.

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