State associations with the cortisol awakening response in healthy females

Tobias Stalder a, b, Phil Evans a, Frank Hucklebridge c, Angela Clow a, *

a Department of Psychology, University of Westminster, 309 Regent Street, London W1B 2UW, UK
b Department of Psychobiology, TU Dresden, 01062 Dresden, Germany
c Department of Human and Health Sciences, University of Westminster, 115 New Cavendish Street, London W1M 8JS, UK

Received 20 October 2009; received in revised form 14 February 2010; accepted 21 February 2010

KEYWORDS
Cortisol awakening response; CAR; Saliva; Human; State; Intra-individual; Psychosocial; Sleep

Summary The current study examined intra-individual relationships between the cortisol awakening response (CAR) and state sleep-related and psychosocial variables in a pooled design study. 12 healthy female participants (age range: 22–41 years) were examined on 12 study days each, occurring at three-day intervals. Quantitative diaries capturing state sleep-related and psychosocial variables 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 dummy-variable linear regression models. State variability in the CAR (area under increase curve; AUCI) was found to be inversely related to simultaneous variability in awakening time (β = −.29, p < .005) and positively related to variability in adverse psychosocial states of stress (β = .22, p < .01) and tension (β = .32, p < .001) measured 45 min post-awakening. In addition, levels of the CAR were also found to decrease linearly over the study period across participants (β = −.19, p < .01) and this time trend could not be explained through a relationship between the CAR and any of the examined variables. The results are discussed within the context of previous evidence and potential implications for cross-sectional research are highlighted.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

The cortisol awakening response (CAR), the rapid increase in cortisol levels following morning awakening, has increasingly been used as a measure in psychobiological research. This has led to evidence showing the CAR to be associated with a wide range of variables in the psychosocial and health domains (see Fries et al., 2009). Whilst this indicates the principal significance of the CAR as an important component of endocrine activity, to date firm knowledge about the exact nature of associations with the CAR is still scarce. A particular reason for this is that results between studies examining the same or similar constructs have frequently been inconsistent (Clow et al., 2004; Fries et al., 2009). Given this situation, it is of interest to establish potential reasons for these inconsistencies.
With regard to this, an important factor may be the considerable amount of state variability in the CAR which has recently been reported. Hellhammer et al. (2007) applied structural equation modelling on CAR data obtained over six consecutive days and found that single day measurements were determined to a greater extent by state-specific factors (61–82%) than by stable, trait-like characteristics (15–37%; Hellhammer et al., 2007). In line with this, we have recently reported results from a longitudinal case study which further illustrated the extent of state variability in the CAR. In this study, the case participant showed a maximal CAR almost 10 times the magnitude of his smallest CAR (Stalder et al., 2009).

One implication of the considerable state variability in the CAR is the possibility that state-related factors might confound cross-sectional CAR research (Hellhammer et al., 2007; Stalder et al., 2010). State variability in the CAR is unlikely to be random and, indeed, evidence is accumulating showing associations between state changes in the CAR and simultaneous changes in situational variables (reviewed below). Cross-sectional research frequently compares CAR profiles of groups examined under very different situational circumstances (e.g. hospitalised clinical groups vs. home-based controls). This makes the interpretation of findings difficult since group differences in the CAR could not only result from different trait characteristics of the groups but could also be related to state differences related to the circumstance under which the CAR is measured. A prerequisite for reducing or preventing the possibility of such confounding influences on cross-sectional research is detailed knowledge of state variables associated with intra-individual variability in the CAR.

A second implication is that examination of intra-individual variability in the CAR provides an opportunity to increase fundamental understanding of the CAR as it is reasonable to assume that this variability fulfils one (or more) adaptive function(s) for the individual. Unlike cross-sectional research, where researchers are faced with the challenge of teasing out state- and trait-related influences on manifestations of the CAR, examining the CAR repeatedly within an individual means that trait characteristics are kept stable and findings can be more clearly attributed to associations with state changes.

To date, only a small number of studies have examined the nature of state variability in the CAR. In order to examine state associations between the CAR and sleep-related variables, both Federenko et al. (2004) and Williams et al. (2005) have used shift work as a model of naturally induced state variability in awakening times. Their results show that both nurses as well as London underground railway workers exhibited elevated CAR profiles on early compared to later shifts (Federenko et al., 2004; Williams et al., 2005, respectively). This is consistent with cross-sectional findings of inverse associations between awakening time and the CAR reported by some (Edwards et al., 2001; Kudielka and Kirschbaum, 2003) but not all researchers (Pruessner et al., 1997; Wüst et al., 2000b; Brooke-Wavell et al., 2002; Kunz-Ebrecht et al., 2004). Besides the CAR, Williams et al. (2005) also reported reduced cortisol levels on awakening in earlier shifts, which is in line with a strong positive association between awakening time and waking cortisol seen in a longitudinal case study (Stalder et al., 2009).

Associations between state changes in psychosocial variables and the CAR have also been examined in a small number of studies. Two studies have suggested that adverse psychosocial states of loneliness/sadness/threat (Adam et al., 2006) and reduced happiness (Stalder et al., 2010) on the day prior to a study day were related to a more pronounced CAR the following day. With regard to psychosocial states on the study day, state changes in the level of arousal at 45 min post-awakening were found to be positively associated with changes in the CAR (Thorn et al., 2004, 2009; Stalder et al., 2010). Another line of intra-individual research is linked to the recent proposition that the CAR might be related to ‘booting’-like processes following morning awakening, including the activation of prospective memory representations related to anticipations of the forthcoming day (Wilhelm et al., 2007; Fries et al., 2009). This notion has received some tentative support by the finding of a more pronounced CAR on days for which more obligations and less leisure time were anticipated (Stalder et al., 2010) as well as by evidence of elevated CARs on weekday compared to weekend days (Kunz-Ebrecht et al., 2004; Schlotz et al., 2004; Thorn et al., 2006; but see Kudielka and Kern, 2004; De Weerth and Buitelaar, 2005; Okun et al., 2009).

However, previous research in this area has mostly relied on a relatively small number of consecutive study days per person, setting a natural limit to the amount of variability that can be expected. Although the recently published case study by Stalder et al. (2009, 2010) was an attempt to examine state associations with the CAR over a more protracted period of time, its generalisability was limited by its reliance on a single subject. The current study aims to extend this research by examining state associations with the CAR in a somewhat larger sample whilst maintaining a considerable intra-individual component per participant. In order to fully capture potentially relevant states, prior- and current-day measurements of sleep-related and psychosocial state and anticipatory variables are applied. In addition, sampling days are carried out on non-consecutive study days to maximise the amount of observed variability. Finally, since variability estimates of Hellhammer et al. (2007) were only based on a six-day period, which leaves the possibility that trait-specificity estimates in this study might have also reflected parts of slower moving state components, the current study also aims to corroborate these important findings in research covering longer time periods per participant.

2. Method

2.1. Participants

12 female participants took part in the study. Mean age of participants was 29 years, ranging from 22 to 41 years. Participants were either employed at the university (four participants) or students (mostly post-graduate). Recruitment was conducted on the basis that participants were taking no medication (other than oral contraceptives), were not suffering from any chronic or acute illness and were not pregnant at the time of recruitment. Two participants reported being regular smokers but agreed to refrain from smoking during the post-awakening period of study days.

Please cite this article in press as: Stalder, T., et al., State associations with the cortisol awakening response in healthy females. Psychoneuroendocrinology (2010), doi:10.1016/j.psyneuen.2010.02.014
2.2. Procedure

The study was carried out on a total of 12 study days per participant, with study days occurring every third day. This resulted in a -1 month study period per participant which fell between the spring—summer months of April—August. On the evening prior to each study day, participants received a standardised reminder via text-messaging to which participants responded. Before going to bed in the evening, participants attached a wrist-worn motility meter (see below for details) and filled out a state diary (evening entry, see below). Saliva samples were taken immediately on awakening and at 15, 30, and 45 min post-awakening. During this period participants were not allowed to take anything by mouth, other than water, or to brush their teeth, to avoid abrasion and micro-vascular leakage. After taking the last saliva sample at 45 min post-awakening, the participant again filled out a state diary (morning entry). Participation in the study was rewarded with a £30 shopping voucher. The study protocol was approved by the ethics committee of the University of Westminster and conducted in accordance with the Declaration of Helsinki.

2.3. Materials, cortisol analyses and psychosocial measures

Participants were provided with a study pack containing fully standardised written instructions and 12 re-sealable study bags, each containing a record sheet, a state diary (see below) and four pre-labelled Salivettes (Sarstedt Ltd., Leicester, England). Motility readings from wrist-worn actigraphy devices (Actiwatch Score, Cambridge Neurotechnology, Cambridge, England) were used to obtain an objective check on participants' awakening time (as in Dockray et al., 2008). These devices use piezoelectric sensors to record movement intensity and have been validated against polysonmography (Lichstein et al., 2006). Cortisol analyses were carried out using a standard enzyme-linked immunosorbsent assay protocol (Salimetrics, USA). Intra- and inter-assay variations were both below 10%. Cortisol concentrations are reported in nmol/l.

The same state diary system as in Stalder et al. (2009, 2010) was used. In brief, the diary system consists of evening and morning entries, which both use the same items adapted to specific targeted times and time periods. Diary recordings of sleep-related information are based on the Pittsburgh Sleep Diary (Mork et al., 1994). On the evening before a study day, bedtime was recorded. In the morning entry, time of awakening, number of nocturnal awakenings and mode of awakening (‘alarm clock/radio’, ‘someone whom I asked to wake me’, ‘noises’, or ‘just woke’) were recorded. The latter was later re-coded as a dichotomous variable of ‘externally induced awakenings’ (first three items) vs. ‘spontaneous awakenings’ (last item). In addition, a record of participants’ perceived sleep quality was obtained via a 100 mm visual analogue scale (VAS) where 1 mm was ‘very bad’ and 100 mm was ‘very good’.

Psychosocial state variables were also assessed via 100 mm VAS and included Mood (very tense vs. very calm; reported in the direction of tension), Alertness (very sleepy vs. very alert), Happiness (very sad vs. very happy), as well as ‘How much of today have you spent fulfilling obligations (work or other)?’ (nothing at all vs. very much) and ‘How much of today have you spent doing leisure activities?’ (nothing at all vs. very much). These items were assessed on the evening prior to the study day as a retrospective assessment for this day (‘Feelings about today.’), as well as on the study day morning, 45 min post-awakening as momentary state measures (‘Feelings now.’) and as measures of anticipations regarding the day ahead (‘Feelings about the coming day.’). In addition, the Stress Arousal Checklist, which distinguishes between feelings of unpleasantness/pleasantness, i.e. hedonic tone: stress, and wakefulness/drowsiness or vigour: arousal (SACL, Mackay et al., 1978) was also completed as part of the morning diary at 45 min post-awakening to assess momentary state stress and arousal.

2.4. Data exclusion and statistical analysis

Data were excluded if the participant self-reported a delay in saliva sampling >10 min or if a difference >10 min between self-reported awakening time and the objective proxy of awakening time (based on actigraphy data) was found. On the basis of the latter criterion, data from two participants on one study day each were excluded from analyses. In addition, since there is evidence suggesting that the CAR is a morning-specific phenomenon (e.g. Leproult et al., 2001), data was excluded on days when participants had woken up later than 11:00 h. This led to the further exclusion of data from one study day of one participant. Three participants, each on one study day, also provided insufficient saliva for cortisol assay, resulting in a total of 138 study days remaining for analyses.

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. Significance probabilities for within-days terms in the ANOVAs were corrected for sphericity violation where appropriate by using the Greenhouse—Geisser method. For the examination of associations with cortisol data, two measures were used: the level of cortisol on awakening, which describes the endpoint of the pre-awakening cortisol increase, was quantified as cortisol concentrations in the first awakening sample (S1). The area under the cortisol curve with respect to increase (AUCi; calculated as suggested by J. Pruessner et al., 2003) was used as the measure of the CAR (i.e. the dynamic of the response). Prior to further analyses, estimates of the extent to which variability in the two cortisol measures, S1 and AUCi, could be divided into a person-specific (trait) and an occasion-specific (state) component were obtained. To do this, first two categorical variables were computed, reflecting the study participants and the measurement occasion (i.e. study days). A univariate ANOVA was then carried out in which these two variables were entered as fixed factors. To determine respective percentage rates of variability-specificity, the resulting sum of squares for the respective variable was divided by the total sum of squares for the corrected model and then multiplied by 100. Person-specific variability was calculated as reflecting the relative sum of squares of the participant variable. Occasion-specific variability was calculated as the added sums of squares of the occasion variable and the participant x occasion interaction component.

The data obtained in the current study combined cross-sectional- (12 participants) with time series- (12 measurements per participant) components. Data from such
pooled- or panel designs can principally be examined using standard ordinary least squares regression models; however, some additional statistical steps are warranted. Firstly, as the interest in the current study was in examining associations within individuals, inter-individual variability needed to be accounted for in regression models to allow accurate specification of intra-individual effects. This was done by creating \( N \) (of cross-sections – 1) dummy variables with ‘0’ representing the absence and ‘1’ representing the presence of a particular cross-section. Dummies were entered into models as regressors and maintained irrespective of whether or not they reached significance levels as predictors (Sayrs, 1989; Gujarati, 2003).

The second factor that needed to be considered in the current analysis was related to the fact that data of individual participants consisted of time series. Here, a potential danger lies in obtaining spurious results from regression analyses of variables which share a linear time trend but are not otherwise related to each other (Gujarati, 2003). In order to protect against this possibility, a variable representing the linear sequence of measurement days was created and its association with all variables was examined by entering it as a regressor in dummy-regression models, with significant findings indicating the presence of a linear time trend. If a linear time trend was found for a variable, further analyses with this variable were confirmed in analyses in which the linear time trend variable was entered as a regressor in the dummy-regression model.

3. Results

3.1. Descriptive information and relationship between cortisol measures

Table 1 shows descriptive information of cortisol measures for all participants. The ANOVA of changes in cortisol values over the post-awakening period across participants and days showed a significant effect of sampling time \( F(2.0,274.5) = 74.47, p < .001 \), partial \( \eta^2 = .35 \), demonstrating the expected cortisol awakening response. No increase in cortisol levels, from \( S1 \) to later samples, of at least 2.5 nmol/l (a secretory episode; Wüst et al., 2000b), was seen on 23.4% of study days.

Considerable variability in \( S1 \) and \( AUC_i \) values was seen both between- and within participants. The analysis of trait and state-specificity of variability revealed that the total variability for \( S1 \) (sum of squares of the corrected model: 52.56) divided into 35.88% (18.86) trait-specific- and 64.40% (33.85) state-specific variability. The total variability for the \( AUC_i \) (sum of squares of the corrected model: 63646.8) divided into 34.95% (22243.0) trait-specific and 64.15% (40829.3) state-specific variability.

The net dummy-variable regression model was found to be highly significant for both \( S1 \) (adjusted \( R^2 = .30, p < .001 \)) and the \( AUC_i \) (adjusted \( R^2 = .30, p < .001 \)). Hence, dummy variables were included in all further regression models, unless specified. The analyses of the development of cortisol measures over the study period revealed no linear time trend for \( S1 \) (\( \beta = .03, p = .67 \)). However, an inverse linear time trend was found for mean levels of the \( AUC_i \) (\( \beta = -.19, p < .01 \)), indicating that cortisol awakening responses across participants tended to decrease over the study period. Dividing \( AUC_i \) values into the first and the second half of the study period (each six days) showed that mean \( AUC_i \) values decreased by 34.63% (first half: 176.49; second half: 115.37) across participants. This time trend in the \( AUC_i \) could not be explained by associations with any of the other examined variables. The variable of state tension at 45 min post-awakening was also found to follow a linear time trend (\( r = .22, p < .01 \)); however, since this time trend was positive and state tension was also found to be positively related to the \( AUC_i \) (\( \beta = .22, p = .01 \); see below), this variable could not be responsible for the inverse time trend in the \( AUC_i \). As a protection against the possibility of obtaining spurious results, further analyses with the \( AUC_i \) were confirmed in analyses in which the linear time trend variable was controlled for.

An inverse relationship was found between \( S1 \) and the \( AUC_i \) (\( \beta = -.44, p < .001 \)). This association also emerged when effects of linear time trends were accounted for (\( \beta = -.43, p < .001 \)).

3.2. Cortisol measures and sleep-related variables

The mean (±standard deviation, SD) difference between self-reported awakening times and the objective proxy of awakening time (based on actigraphy data) was 3 min 31 s (±2 min 37 s). As there were only minor differences between the two measures, self-reported awakening times were used in further analyses. Descriptive information of sleep-related variables is presented in Table 2, columns 2—5. In addition to the information provided there, across participants an equal share of externally induced and spontaneous awakenings on 50% of study days was reported.

Relationships between sleep-related variables and cortisol measures are shown in Table 2, columns 6 and 7. A significant inverse relationship was found between time of awakening and the \( AUC_i \), and this relationship remained significant controlling for linear time trends. No further significant relationships between sleep-related variables and cortisol measures emerged. This included the analyses of reported mode of awakening which revealed no significant differences between externally induced and spontaneous awakenings with regard to \( S1 \) (\( F(3,123) = .51, n.s. \)) and the \( AUC_i \) (\( F(3,123) = .78, n.s. \)).
3.3. Cortisol measures and psychosocial state variables

Table 3 (columns 2—5) provides descriptive information of psychosocial state variables. Measures of obligations and leisure activities were found to be significantly inversely related with regard to both retrospective prior-day assessment ($r = - .82$, $p < .001$) as well as anticipations for the study day ($r = - .75$, $p < .001$). These variables were thus collapsed into a single variable obligations/no leisure for both time periods by computing a single principal component score reflecting their common variance.

Table 3 (columns 6 and 7) also shows results of associations between psychosocial state variables and cortisol measures. The level of S1 was found to be significantly positively related to anticipations of tension for the study day measured 45 min post-awakening. Significant positive associations with the AUCI were found for the reported level of tension and the reported level of stress at 45 min post-awakening on the study day. Re-analyses accounting for linear time trends confirmed associations found for both variables. When the two variables were entered simultaneously into a dummy-regression model the reported level of stress lost its statistical significance ($\beta = .10$, n.s.), due to the fact that the two variables were strongly positively related to each other ($r = .56$, $p < .001$). No other significant relationships between psychosocial state variables and the AUCI were found. However, when linear time trends were accounted...
for, a non-significant trend for an inverse relationship between prior-day happiness and the AUCI was seen ($\beta = -.13$, $p = .98$).

### 3.4. Overall model for AUCI

Since variables of different categories were found to be significantly related to the AUCI, it was further examined whether these variables predicted the AUCI independently of each other. Table 4 shows the results of dummy-regression models in which variables previously found to be significantly associated with the AUCI were entered simultaneously.

In the model of original time series, each of the three variables was a significant predictor of the AUCI. These variables added a further 21.8% of explained variability in the AUCI to the dummy-variable model. The picture emerging from confirmatory analyses accounting for linear time trends was very similar, with the detrended time series of the three variables adding a further 25% of explained variability to the pure dummy-regression model. To determine the individual predictive impact of the level of state tension, additional analyses were conducted in which S1 and awakening time were first entered into the model and state tension added subsequently. This showed that state tension explained a further 4.6% of variability in the AUCI when original time series were used and 8.2% in a model of detrended time series.

### 4. Discussion

In the present study we set out to examine patterns of state variability in the CAR and its relationship to simultaneous variability in state variables of interest in a pooled design study. On a descriptive level, our results corroborate the notion that the CAR is a highly state-dependent phenomenon, with estimates of occasion- and person-specificity of variability closely mirroring values reported by Hellhammer et al. (2007). Our results also support previous evidence of an inverse association between state changes in awakening time and the CAR and indicate that self-reported psychosocial states of tension and stress covary with changes in the CAR.

The current results corroborate the evidence of Hellhammer et al. (2007) which suggest that between 61% and 82% of a single day measurement of the CAR, quantified as AUCI, is determined by state-specific factors. The current results mirror these estimates closely, suggesting that across participants 64% of the variability in the CAR was state-specific. This is particularly important given that variability estimates in the current study were based on a longer time period than those of Hellhammer et al. and thus the likelihood that trait estimates might have been influenced by slower moving states was reduced. Clearly, the examined time period of ~1 month per participant was still limited and it is likely that very slowly moving states, e.g. related to circannual periodicity in light levels, which are known to affect the CAR (Thorn et al., 2004), were not reflected in the current estimates. Nevertheless, the similarity of the current results with those of Hellhammer et al. suggests that the largest part of situational variability can be captured in relatively short time periods of approximately one week per participant.

With regard to the nature of the state associations with sleep-related variables, the main finding of the current study was an inverse relationship between time of awakening and the CAR. This finding is in line with previous evidence from intra-individual (Federenko et al., 2004; Williams et al., 2005) and some inter-individual (Edwards et al., 2001; Kudielka and Kirschbaum, 2003) research. The awakening time finding in the current study was independent of concurrent changes in waking cortisol levels and psychosocial states, suggesting that a genuine relationship between awakening time and the CAR was observed. On the other hand, the current results did not reveal evidence for a strong positive association between awakening time and S1 which we had previously reported from a longitudinal case study (Stalder et al., 2009).

In line with most previous research (but see Backhaus et al., 2004; Lasikiewicz et al., 2008), the level of cortisol on awakening or the CAR were not found to be related to bedtime, sleep duration, sleep quality, number of nocturnal awakenings or mode of awakening. Thus, the current results further add to the evidence suggesting that time of awakening is an important variable to be considered in research on the CAR, both in terms of inter- as well as intra-individual variability.

A central interest of the current study was in examining covariance between changes in post-awakening cortisol measures and simultaneous changes in psychosocial state and anticipatory variables. Here, the arguably most important finding was that, across participants, variability in the psychosocial states of stress and tension measured 45 min post-awakening was positively associated with variability in the CAR. This finding is at variance with results from previous
intra-individual research, which has mostly shown the CAR to be related to state arousal but not state stress (Thorn et al., 2004, 2009; Stalder et al., 2010). On the other hand, the current findings can be seen as concurring with results of Schlotz et al. (2004) of greater CAR elevations on weekdays as compared to weekend days in more chronically stressed individuals as well as with cross-sectional evidence of an elevated CAR in chronically stressed individuals (Schulz et al., 1998; Wüst et al., 2000a; M. Pruessner et al., 2003; but see Pruessner et al., 1999; Thorn et al., 2006). The disparity between these findings is difficult to explain, particularly since CAR association with state tension at 45 min post-awakening did not extend to study day anticipations of tension. Irrespective of this, the current findings again highlight the possibility of state-related confounding influences on cross-sectional CAR research, which might occur when examined groups, in addition to their primary trait characteristic, also differ with regard to their momentary states of stress or tension. Different participant groups might further interpret the CAR measurement situation as either more or less stressful/tense and this could lead to false conclusions with regard to the group’s CAR under normal conditions. This highlights the importance of considering the possibility of state-related confounding in cross-sectional CAR research.

The current findings did not reveal associations between study day anticipations and the CAR, and thus preliminary evidence suggesting that an elevated CAR is found on days anticipated to be busier (Stalder et al., 2010) was not corroborated. Hence, the current results are at variance with part of the proposition that the CAR covaries with prospective memory activations related to anticipations for the day ahead (Wilhelm et al., 2007; Fries et al., 2009). Whilst no associations between study day anticipations and the CAR were seen, elevated levels of cortisol on awakening were found on days when participants anticipated being more tense. Since such an association was not hypothesised, detailed interpretation of this finding should be cautious and await corroboration by future research.

Another finding of the current study is that across participants’ mean levels of the CAR were found to decrease linearly over the study period. Interestingly, this trend could not be explained through a relationship with any of the other examined variables, e.g. sleep-related variables or psychosocial states. The, arguably, most likely explanation of this finding is that the CAR was related to a variable that changed systematically over the examined period but was not captured in the current study. It is conceivable that an initial reaction to the novelty of the study situation might have affected CAR results; however, in this case it might be expected that such an effect would have, at least partly, been captured by the psychosocial state measures employed. An alternative explanation is that a gradual change in an environmental factor, that was shared between participants and related to the CAR, was responsible for this result. Again, a potential variable that comes to mind is the level of light (see Thorn et al., 2004). However, given that the study was carried out between the spring/summer months of April to the beginning of August, during which time light levels in England mostly increase, and given that light is assumed to be positively associated with the CAR, this is unlikely. Irrespective of the explanation of this finding, the possibility of linear time trends has implications for longitudinal CAR research, e.g. intervention studies, and highlights the importance of using adequate counterbalancing techniques in such research.

Participant non-adherence to the study protocol 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). The current study aimed to address this issue by obtaining an objective check on participants’ awakening time from wrist actigraphy readings. Whilst this strategy has been used previously (Dockray et al., 2008), it is clear that it cannot protect against participants reporting their awakening times correctly but delaying the subsequent saliva sampling relative to awakening. Since it is conceivable that the extent of non-adherence might covary with situational characteristics — e.g. non-adherence seems to be more prevalent on weekend than on weekdays (Thorn et al., 2006) — the possibility of non-adherence-related confounding influences on the current results cannot be excluded. Hence, future research should ideally electronically monitor sampling times (e.g. as in Kudielka et al., 2003) as well as obtain an objective proxy of participants awakening time.

In conclusion, the results of the current pooled design study corroborate previous evidence and illustrate the considerable extent of state variability in the CAR. It adds to this body of literature by identifying the nature of the pertinent state variables that affect day-to-day variability in this measure. Whilst a range of sleep-related variables have once more been shown not to influence the CAR an inverse association between awakening time and the dynamic of the CAR has been confirmed. The current findings also highlight the influences of state psychosocial variables on variability of the CAR as well as the decreasing dynamic of the CAR following repeated sampling (which in this study could not be accounted for by any of the measured state variables). These data corroborate the need to take account of systematic differences in such state variables when exploring group differences in the CAR and question the methodology of relying upon single day sampling in such cross-sectional research. It is possible that such accounted for state variation in the CAR has contributed to the inconsistent literature in this field.

Role of the funding source

This project was supported by internal funding from the University of Westminster.

Conflicts of interest

There are no conflicts of interest

References


Please cite this article in press as: Stalder, T., et al., State associations with the cortisol awakening response in healthy females. Psychoneuroendocrinology (2010), doi:10.1016/j.psyneuen.2010.02.014


