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The cortisol awakening response predicts a same-day index of executive function in healthy young adults

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Contributions

All authors have contributed to the design of the study, analysis of data, and preparation of the manuscript. R. Law has conducted the data collection. All authors have approved the final article.

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Abstract

The cortisol awakening response (CAR) is associated with various aspects of cognition, including executive function, in older adult and clinical samples. However, the association between these variables in the healthy functioning population is not well understood due to the limited number of appropriately controlled studies. This study explored the association between the CAR and a set shifting index of executive function in 55 (44 females) healthy participants aged 20.2±3.0 years. Notoriously, assessment of the CAR from self-collected saliva samples within the domestic setting is subject to sample timing error, so electronic monitoring of both awakening and sampling times were employed. Participants attended the laboratory in the afternoon of CAR assessment for testing on the Attention Switching Task of the CANTAB neuropsychological testing battery. A positive association was found between CAR magnitude and attention-switching performance in the afternoon of the same day. This was independent of known relevant CAR covariates, but only evident in CAR data collected without delay exceeding 8 min post-awakening. These findings offer insight into a potential role for the CAR in modulating cognitive functions associated with the pre-frontal cortex.

Keywords

CAR; saliva; pre-frontal cortex; attention-switching task; executive function; cognition.

Highlights

- Explored the CAR and same day attention switching in 55 healthy young adults
- Positive association between CAR and attention switching performance
- Associations only evident where CAR sampling was accurate to within 8 mins

- Results indicate a role for the CAR in healthy pre-frontal cortex function

1 Introduction

The cortisol awakening response (CAR) is the rapid increase in cortisol concentrations within the first hour after awakening from night-time sleep, peaking at about 30-40 min post-awakening (Pruessner et al., 1997). The most prominent theory for the function of the CAR is that it provides an allostatic 'boost' upon awakening (Adam et al., 2006; Clow et al., 2010; 2014; Fries et al., 2009; Law et al., 2013). Accumulating evidence suggests that the CAR is associated with cognitive functions dependent upon brain regions with high densities of glucocorticoid receptors, such as the hippocampus and pre-frontal cortex (Buchanan et al., 2004; Evans et al., 2012; Law et al., 2015; Wolf et al., 2005; Zhang et al., 2015). It has been speculated that the CAR is a product of the complex regulatory influences of these brain regions on adrenocorticotropic hormone and cortisol secretion around the time of awakening (Fries et al., 2009; Clow et al., 2010). Further, there is accumulating evidence to show that the circadian rhythm of circulating glucocorticoids promotes the alignment of central and peripheral cellular clocks of the circadian system, and that this is related to fluctuations in arousal and cognition (for review, see Oster et al., 2016). It has therefore been suggested by the authors that the CAR may serve as a time-of-day marker, synchronising circadian rhythms in peripheral cellular clocks within the body and brain under the regulatory influence of the suprachiasmatic nucleus of the hypothalamus, given that the suprachiasmatic nucleus modulates cortisol secretion and the CAR is the most prominent, dynamic and variable part of this rhythm (Clow et al. 2010, 2014; Law et al., 2013).

The evidence for a positive association between CAR and cognition is strictly correlational in nature (Buchanan et al., 2004; Evans et al., 2012; Law et al., 2015; Wolf et al., 2005; Zhang et al., 2015) and therefore three possible explanations exist: that the CAR modulates cognition, that cognition modulates the CAR, or that both fluctuations in CAR and cognition are influenced by some common factor (for example, integrity or functioning of the hippocampus or pre-frontal cortex). There are well-established circadian and sleep-related fluctuations in cognitive functions (Schmidt et al., 2007; Manly et al., 2002), but the mechanisms underlying these rhythms have not been fully identified. What is known is that the suprachiasmatic nucleus of the hypothalamus synchronises circadian rhythms in brain and body by a range of signalling methods, including the modulation of hormone secretion. If, as the authors have proposed, a function of the CAR is to act as a circadian signal to synchronise peripheral clocks, then CAR prediction of some aspects of cognition in the afternoon of the same day would be expected. This is perhaps especially probable in those brain regions with a greater density of cortisol receptors and well-established functional relationship with glucocorticoids, such as the hippocampus and pre-frontal cortex.

Most of the CAR and cognition studies have focused upon associations with hippocampal functions, and the positive association of CAR magnitude with hippocampal integrity and associated memory functions is now reasonably well established (e.g. Bäumler et al., 2014; Buchanan et al., 2004; Pruessner et al., 2007; Rimmele et al., 2010; Wolf et al., 2005). It has been demonstrated that the CAR is attenuated or even absent in cases of hippocampal destruction and severe amnesia (Buchanan et al., 2004; Wolf et al., 2005), and that pharmacologic suppression of the CAR inhibits hippocampus-dependent declarative memory (Rimmele et al., 2010). CAR magnitude has also been shown to be positively associated with episodic memory in healthy adults (Ennis et al., 2016), and prospective memory performance in children (Bäumler et al., 2014). Recent evidence also suggests that the circadian rhythm of glucocorticoids may support memory function by regulating

plasticity in the hippocampus (see Oster et al., 2016). While such associations with declarative memory have been investigated in some detail, considerably less is known about associations with pre-frontal (executive) functions.

The frontal cortex influences cortisol secretion via regulation of the hypothalamic-pituitary-adrenal (HPA)-axis (Lupien et al., 2009), and this relationship is reciprocal, such that endogenous glucocorticoids modulate the cognitive functions of the pre-frontal cortex (Mizoguchi et al., 2004). However, the relationship between cortisol secretion and executive function is relatively complicated, not least because executive function is a non-unitary and fractionated concept, including a range of high-level cognitive processes dependent on several separate pre-frontal cortex structures, and responsible for control and organisation of other cognitive functions (Miyake et al., 2000; Gilbert and Burgess, 2008; Diamond, 2013). Consensus has not yet been reached on exactly what executive function comprises (Gilbert and Burgess, 2008), but perhaps the best evidence is for it consisting of three moderately related functions: mental 'set shifting' (also known as 'cognitive flexibility') which is the ability to switch between task demands, 'updating' or working memory functions, and 'inhibition' which includes both cognitive and behavioural inhibitory processes (Miyake et al., 2000; Diamond, 2013).

It has been demonstrated with some measures of set shifting and response inhibition that there is a positive association with acute increases in exogenous glucocorticoids (Dierolf et al., 2016; Shields et al., 2015; Vaz et al., 2011). Furthermore, acute increases in endogenous cortisol are associated with immediate and short-term increases in set shifting, measured as switch cost (the delay brought about by responding to a switch in task demands for reaction time performance; Dierolf et al., 2016). However, inconsistent results have been reported from other studies in which the timing of the task (differentiating between rapid non-genomic, and slower genomic effects of cortisol secretion; see Shields et al., 2015) and the

specific index used to measure executive function have varied. For example, both Wingenfeld et al. (2011) and Vaz et al. (2011) have reported that the association of exogenous cortisol and set shifting is no longer apparent if measured more than an hour later. Although, it should be noted that the former measured set shifting as reaction times on accurate responses (which does not assess the cost of task-switching on performance, or what would typically be considered set shifting), and both of these studies employed very small samples (n≤20).

With respect to the CAR, positive associations have been reported for CAR magnitude with both working memory and attention-switching performance in older adults (Almela et al., 2012; Evans et al., 2012). In a recent study, Shi et al. (2018) further suggested that larger CAR magnitude is associated with improved response inhibition (using a Go/No go paradigm) in the afternoon of the same day. But negative associations with task updating, speed of memory, error monitoring, and serial sequence learning have been reported in other samples (Hodyl et al., 2016, Maldonado et al., 2008; Oosterholt et al., 2016; Zhang et al., 2015). Similar inconsistency has occurred in studies of CAR associations with overall cognitive performance (e.g. Aas et al., 2011; Evans et al., 2011; Labad et al., 2016).

One area in which there has been greater consistency is in studies of the CAR and set shifting (Evans et al., 2012; Law et al., 2015). The first of these studies (Evans et al., 2012) demonstrated that in a sample of older adults (ages 60-91) both trait CAR magnitude and earlier CAR peak were positively associated with Trail Making Test performance measured on a separate day. The second of these studies was a case study by the authors which explored day-to-day variation in the CAR in a healthy young adult male, demonstrating a positive same-day, within-subject relationship between the CAR and attention switching task performance 45 min post-awakening (Law et al., 2015). As both the Trail-making task and the attention switching task can be considered measures of set shifting (Olivera-Souza et al.,

2000), and such tasks have been shown to be dependent upon common pre-frontal cortex mechanisms (Kim et al., 2011), the results of these studies offer consistent support for set shifting measures being linked to the CAR.

The inconsistency of results from other studies of CAR and executive function may be due to variation in nature and timing of the cognitive assessments, sample populations, and irregularity in monitoring participant adherence to the CAR protocol (see Stalder et al., 2016). Studies of diurnal cortisol secretion show reduced effect sizes when sampling accuracy is not monitored (Adam et al., 2017), and studies of the CAR are particularly vulnerable to confounding effects of inaccurate sampling if delay exceeds the beginning of the dynamic increase in cortisol section ~8-min post-awakening (Clow et al., 2004; Smyth et al., 2016). Another factor which contributes to confusion around the CAR-executive function association in healthy function is that most of the previous research has sampled from older adult or clinical populations. Relatively few studies have explored this association among healthy functioning young adults and it is yet to be established whether the positive association between CAR and set shifting can be generalised to this population.

In summary, a positive relationship between average CAR magnitude and set shifting has been demonstrated in older adults (Evans et al., 2012), and similar positive relationships have been found in a case study of same morning attention switching (Law et al., 2015). The aim of the present study was to employ best practice methodology as laid out by Stalder et al. (2016) to investigate generalisability of the previously observed CAR association with attention switching in a sample of healthy young participants, in the afternoon of the same day. The primary hypothesis was that CAR magnitude would positively predict same-day attention switching performance.

2 Materials and methods

2.1 Design

Ethical approval was provided by the Institutional Ethics Committee, and analysis of cortisol samples was conducted in accordance with the Human Tissues Act (UK). The design employed was a correlational, naturalistic study across two consecutive weekdays, to explore same-day effects and control for a potential novelty effect of testing on a single day. Participant adherence to the CAR sampling protocol is recognised as a potential confound in previous studies (for review, see Clow et al., 2004, 2010; Smyth et al., 2013), and therefore the methodology here was in strict compliance with the expert consensus guidelines of Stalder et al. (2016) to maximise accuracy in estimation of the CAR and increase potential comparative value of the present data with that of other compliant CAR studies. Such controls included electronic monitoring of adherence to the sampling protocol using wrist-worn actigraphy and medical event monitoring system (MEMS) caps.

2.2 Participants

Participants were students recruited from the University of Westminster (UK) Psychology Department's Research Participation Scheme (RPS), in addition to volunteers from the academic community. Participants were recruited between September and February, during normal study and outside of the examinations period. Participants self-reported being in good health, being non-smokers, and being free from medication. The initial sample consisted of 55 participants, however complete data for one participant was excluded due to delays of >15 min between awakening and collection of the first sample on both days. The final sample therefore consisted of 54 healthy participants (44 females, 10 males), with a mean age of 20.2 (SD = 3.0) years. Of these, 48 were non-smokers, 5 were ex-smokers and 1 was an occasional smoker. Thirteen participants were using oral contraceptives at the time

of participation, while no participants were taking any other medications known to effect cortisol secretion. Those recruited from the RPS scheme received research participation time. Participants received no other incentive to participate.

A priori at the time of design conception, a typical sample size for published CAR studies was adopted, with reported effects sizes for this type of study converging on a minimum of .09 (R2) but more often exceeding this. Our design characteristics were typical of such comparator studies. Power estimation is meaningless without attention to the reliability and validity of key measures. It is of the essence to emphasise the precise objective electronic monitoring checks on timings of cortisol measurement in this study. In terms of assuring adequate power, a balance was sought therefore in optimizing the relative contributions of additional costs of achieving precision measurement and total number of participants.

2.3 Materials

Saliva samples were obtained using 'Salivettes' (Sarstedt Ltd., Leicester, UK). The cortisol assay procedure was carried out in the Psychophysiology and Stress Research Group (PSRG) laboratory at the University of Westminster. Samples were frozen at -20 C within 1 day of collection and stored at this temperature until analysis. Samples were thawed and centrifuged at 1,500 g (at 3,000 rpm) for 10 min, after which cortisol concentrations were determined by Enzyme Linked ImmunoSorbent Assay (ELISA; Salimetrics, State College, PA). All saliva samples, controls and standards were assayed in duplicate and both intra- and inter-assay coefficients of variation were below 10% in all cases. The limit of detection of the assay was 0.33 nmol/L. Undetectable samples (five in total, from two participants) were treated as missing data, and all other cortisol concentrations were included in the final analysis.

Participant adherence to the CAR sampling protocol was monitored using wrist actigraphy (Actiwatch, Phillips, UK), along with MEMS caps (The Aardex Group, Sion, Switzerland). Participants were also required to complete a sampling time self-report sheet on each morning for verification of self-report with electronic measures. Clock times on the actigraphy and MEMS devices were carefully checked for consistency with the participants' time keeping devices (e.g. wristwatch or mobile phone) during the pre-study one-to-one briefing, to ensure inconsistency in these values would not confound later comparisons between electronic monitoring and self-report. Written study guidelines were also provided in the form of a participant checklist to ensure participants had a clear understanding of the study schedule. Actigraphy-recorded awakening times were scored by the human eye, in line with the recommendations of Boyne et al. (2013). Further, at least 10% of these researcher-scored Actiwatch-measured awakening times were cross-checked among co-authors to ensure consistency.

Evening and morning diaries were included to measure potential confounding variables: participants' self-reported psychosocial states, prior day alcohol consumption and exercise. These consisted of an adapted version of the Pittsburgh Sleep Diary (Monk et al., 1994), measuring levels of perceived obligation, mood and anticipated sleep quality, and the Stress/Arousal Check List (Mackay et al.; 1978). Alcohol consumption was recorded in estimated units of 10ml alcohol, and exercise recorded as sustained moderate to hard exercise of both aerobic and anaerobic nature in the day prior to CAR sampling. These were aided by an attached guide to 10ml units of alcohol in commonly consumed alcoholic drinks, and an adapted version of the perceived exertion scale (Borg, 1981).

Attention-switching performance was assessed using the 'Attention Switching Task' from the Cambridge Neuropsychological Test Automated Battery (CANTAB®; Cambridge Cognition, Cambridge, UK) Eclipse version 5.0.0. This (Stroop-like) task assesses the ability to

manage conflicting information provided by the direction of an arrow and its location on the screen and to ignore task-irrelevant information. The primary outcome measure of interest from the task was switch cost (SC); the same measure as used by Law et al. (2015). This test was administered using Windows operating system on a 15.6 inch touch-screen tablet computer, and was administered by the researcher with strict adherence to version 5.0.0 of the CANTAB Eclipse 5 test administration guide (Cambridge Cognition Limited, 2012). Participants' responses to the task were recorded using a 2-button press-pad connected to the computer.

2.4 Procedure

Prior to data collection, all participants attended a 1-to-1 briefing session with the lead researcher, lasting approximately 30 minutes. This consisted of a detailed verbal description of the procedure, including emphasis on the importance of strict adherence to the CAR sampling protocol and an opportunity to ask questions. All participants were required to complete two consecutive days of testing. Participants were contacted by SMS message in the evening prior to data collection on both days of participation. This SMS message provided a reminder of the sampling procedure. Prior to going to sleep, participants filled in the evening diary to assess their self-reported psychosocial state and time of going to bed, and put on the Actiwatch, which was then worn throughout the night to electronically record sleep duration and awakening times.

Saliva samples were collected immediately upon awakening, and at 15, 30, & 45 min post-awakening by participants chewing on a cotton swab for 1-2 min, and then returning the swab to the Salivette. Participants were instructed to continue with their normal routine throughout the CAR sampling period, apart from those activities that would impair the CAR assessment; protocol instructions were to take nil by mouth other than water, and to refrain

from brushing teeth to avoid abrasion and microvascular leakage. Upon completion of the morning sampling, participants were required to fill out the morning diary, including the adapted Pittsburgh Sleep Diary (Monk et al., 1994) and Stress/Arousal Checklist (Mackay et al.; 1978). Participants then continued with their normal routine, before visiting the university in the afternoon between 12:00 and 15:00 of the same day for the cognitive testing session. Upon arrival for the afternoon session, participants were required to collect one further cortisol sample, using the same sampling procedure (and were advised to follow the morning protocol instructions for at least 30 min prior to taking this sample).

The cognitive test was administered by the researcher in a controlled environment (a private, quiet cognitive testing room) at the University of Westminster. Each participant was tested individually, and the testing was repeated the following day under the same conditions.

2.5 Treatment of data and statistical analysis

SPSS version 24 was used for all statistical analyses. Data were analysed using mixed regression modelling (Blackwell et al., 2006) to examine simultaneously between and within participant sources of variation in attention-switching performance. For the purposes of presentation and consistency with previous research by the authors (Law et al. 2015), the switch cost variable was produced by inverting the 'switch cost' outcome measure from the Attention Switching Task, such that high scores represent better performance. Z-scored SC was then used as the principal covariate. CAR mean increase (MnInc) was calculated, using the simple formula, appropriate for equally spaced samples (s2 + s3 + s4)/ 3 - s1) (Wüst et al., 2000) and a fourth root transformation was performed to counteract positive skewness observed in this composite variable. Both SC and MnInc were standardised for ease of

comparison and interpretation of the results (represented as 'ZSC' and 'ZCAR', respectively), but, for reference purposes, descriptive statistics are presented as raw data.

Session (sampling day) was included as a main factor in the initial model. Sampling delay on each day of CAR assessment was computed using Actiwatch and MEMS cap recordings. Data were treated as accurate where the difference between electronically measured awakening time and timing of the first sample was ≤8 min, as delays greater than this have been shown to result in under-estimated CARs (Smyth et al., 2016). Where delay was >8 but ≤15 min, these data were included in the analyses, and analysed in comparison to accurate data, as per the recommendations of Smyth et al. (2016). Any cases of non-compliance with electronic monitoring, or where electronically measured delay >15 min, were excluded from the analysis, as the typical post-awakening growth-curve is not evident in such data, rendering it unsuitable for real-time modelling (Smyth et al., 2016; Stalder et al., 2016). The remaining sample of (N = 74) CARs came from 41 participants, including 40 from day 1, and 34 from day 2, of which, the 0-8 min (no delay/minor delay) and 9-15 min (moderate delay) groups included 61 and 13 CARs respectively (i.e. 82.9% meeting the criteria for 'no delay/minor delay', and 17.1% meeting the criteria for 'moderate delay'). This delay variable was included as a main factor in a secondary model of the ZSC data, along with ZCAR (but without session). Intercept effects were modelled as both fixed and random. Since only two levels of the repeated-measures variable (session) existed, compound symmetry could be assumed and a random intercept only model was run.

3 Results

3.1 Data and general descriptives

Table 1 presents the descriptive statistics for cortisol samples and composite CAR measures, Attention Switching Task measures, and situational variables as measured on both days. The cortisol data were within the normal range for the age and health status of the sample (Wüst et al., 2000; Stalder et al., 2010). The mean increase in cortisol within the first 45 min post-awakening was 8.59 nmol/l, and the mean peak for cortisol concentrations occurred at 30 min post-awakening.

[Table 1 to be inserted here]

3.2 Modelling of data

3.2.1 The CAR and SC

Table 2 presents zero-order correlations between variables used in the analysis. Initial mixed modelling of ZSC and ZCAR with session indicated that the ZCAR was significantly positively associated with ZSC performance measured on the same day (F (65.237) = 4.280, p= .043), and both session (F (33.814) = 0.204, p= .654) and the session by ZCAR interaction (F (41.512) = 0.001, p= .979) were non-significant. The primary hypothesis, that CAR predicts same day SC, was therefore accepted. Table 3 shows F-ratios and significances for the parameters in this modelled data. In the second model, session was therefore excluded as a fixed effect, but this model investigated whether degree of delay in any way mediated or modulated the overall ZCAR-ZSC relationship. The proportion of data with delay of more than 8-min (but less than 15) was small (17.1% of sample), so, unsurprisingly, no statistically significant effects of delay emerged. Post-hoc exploration of coefficients indicated that where CAR data were measured accurately (i.e. first sample within 8-min of awakening) the association between ZCAR and ZSC remained significant and strong (coefficient (SE)= 0.28 (0.12) p= .025), while the effect was predictably smaller where CAR data was less accurately measured

(9-15 min delay; coefficient (SE) = 0.09 (0.25), *p*= .721). Figure 1 plots the line of fit equations for the predictive relationship between ZSC and ZCAR for accurately and inaccurately collected CAR data. Since SC and CAR were both entered into this model as z-scores, the slope coefficient of 0.28 for accurately measured data indicates a predicted improvement of 21 ms in attention switching performance on the SC element of the Attention Switching Task for every 1 SD increase in magnitude of CAR MnInc.

[Table 3 to be inserted here]

[Figure 1 to be inserted here]

3.2.3 Exploration of potential confounding variables

Further models were produced to examine whether the observed ZCAR-ZSC effect was robust to inclusion of potential confounds. These included afternoon cortisol levels, cortisol levels upon awakening, time of awakening, sex, reaction time scores, prior day exercise, prior day alcohol consumption, contraceptive status, and psychosocial measures. These variables were separately added to the ZCAR-ZSC by accuracy model to achieve a suitable ratio of variables to cases and conserve degrees of freedom. In all models, ZCAR-ZSC remained similarly significant, with a similar effect size, except for the modelling of the interaction between ZCAR and awakening time (where the coefficient, of .23 was similar to the earlier model, but fell short of significance at *p*=.082, with the coefficient for awakening time being -4.45, p=.155) and the model including cortisol levels upon awakening (in which the coefficient again remained similar, at .23, but fell short of significance at *p*=.084, with this variable showing a coefficient value of -.03, p=.200).

4 Discussion

This study explored variation in the CAR and a task-switching index of executive function in healthy young adults. The results indicate that, where CAR data are accurately sampled, the magnitude of the CAR positively predicted attention switching performance as hypothesised. This is the first demonstration of an association between the CAR and attention-switching performance in the afternoon of the same day in healthy young adults, and appropriate CAR measurement criteria were employed (Stalder et al., 2016). The finding is consistent with previous evidence from older adults (Evans et al., 2012), a case study of a healthy young adult male (Law et al., 2015), and a between-subjects study of response inhibition (Shi et al., 2018). The data also suggest that this CAR association with task switching was robust to inclusion of potential confounds such as cortisol levels at the time of cognitive testing and mood states. The results are discussed here in relation to the previous literature, including the convergent findings of studies exploring the importance of sampling time for accurate CAR assessment.

The present finding indicates a relationship between morning CAR magnitude and attention-switching performance in the afternoon of the same day, complementing the previous and within-subject finding of a morning association using the same test in a case study of a healthy young adult male (Law et al., 2015). These results indicate that the association between the CAR and attention switching is not restricted to the immediate post-CAR period. Cognitive functions in humans are influenced by circadian rhythms (Schmidt et al., 2007), and it has been proposed that one role for the CAR may be in the synchronisation of peripheral cellular clocks of the circadian system (Clow et al., 2010; 2014; Law et al. 2013), and if so, associations between CAR and cognition in the same day are to be expected. Such an association between CAR and set shifting can quite conceivably be aligned with the 'Boost' hypothesis of the CAR (Adam et al., 2006; Clow et al., 2010; Fries et al., 2009), and that one specific role for the CAR in this context may lie in allostatic

modulation of cognition (Clow et al., 2014), as better set shifting performance would be an adaptive response to perceived challenge.

The present finding is also consistent with that of Shi et al. (2018), who demonstrated a positive association between CAR and a separate type of executive function (inhibitory performance) in the afternoon of the same day. This suggests that CAR-executive function associations are not restricted to response inhibition but can be observed in attention switching (or set shifting) performance also. Further, it remains unclear if associations found between cortisol and switch cost, or response inhibition (Shi et al., 2018), should be expected to generalise to other executive function measures, or if this is specific to these tasks. Taken alongside the CAR-inhibition association demonstrated by Shi et al., this CAR-shifting association may encourage speculation of a relationship between the CAR and executive function on a more general scale. Such speculation would go beyond what can be interpreted from the present data, but could be investigated in future research.

A distinction is made here between accurately measured CAR data (in which the association was evident), and cases where sampling was delayed beyond 8 min post-awakening (in which it was not). This finding is in agreement with converging evidence from studies of CAR sample timing inaccuracy, showing that failure to control for sampling delay might impact upon reliability of effects (Smyth et al., 2013; 2016). This further emphasises the importance of understanding the effects of sample timing and compliance in future CAR research (Stalder et al., 2016).

For the purpose of clarity, a distinction should be made between the present study and that of Dierolf et al. (2016), who reported a negative association between endogenous morning cortisol across three days and task shifting on a later day. While Dierolf et al. employed a measure of basal cortisol levels (area under the curve with respect to ground; AUCg), in the

present study the CAR was strictly defined as a measure of dynamic increase (see Stalder et al., 2016). Further, the assessment of task shifting on a following day is distinct from the exploration of same-day effects in the present study. As such, the present finding should be interpreted strictly as an association between CAR and same-day SC, while the finding of Dierolf et al. demonstrated a separate association between lower basal cortisol secretion and improved task shifting performance on a following day. These findings would appear to complement, rather than contradict, the present findings since both a positive association for task-switching and CAR and a negative association with basal cortisol are concurrent with previous observations that the healthy functioning cortisol rhythm supports cognition (Oster et al., 2016).

A limitation of the present study is that there were a greater number of females than males in the sample. This was due to the nature of the recruitment and that more females than males volunteered for the study. There are known differences in the CAR between males and females, although the impact of such differences is considered to be small (Fries et al., 2009) and there is no reason to assume that sex differences would confound any association with attention-switching performance. Evidence from previous studies does not suggest that sex has any moderating influence on cortisol-executive function associations, at least in studies of acute, exogenous cortisol levels (Shields et al., 2015). The CAR-SC by accuracy model was also robust to inclusion of sex as a covariate. Nevertheless, the greater representation of females than males here could be considered a potential limitation for the interpretation of the present finding.

It is also important to be cautious in the interpretation of the present results due to their being correlational in nature. It is possible, for example, that the both CAR and task switching could be influenced by a common factor. The mechanisms underlying associations between the CAR and cognition are not well understood, but there is evidence that the CAR

is under complex regulatory influence of several brain regions, including the pre-frontal cortex (Fries et al., 2009; Clow et al., 2010). While it is unclear whether the activity in the pre-frontal cortex which modulates HPA axis activity is the same as that which influences task switching performance, this remains a possibility, as noted in other studies of CAR executive function associations (e.g. Zhang et al., 2015). It is also known that the CAR is influenced by sleep-related variables such as sleep duration and time of awakening (Stalder et al., 2016). Sleep processes can influence both CAR and executive function but are difficult to control for in a naturalistic study beyond the wrist-worn actigragraphical measures applied here. The present findings did not appear to be influenced by sleep and related psychosocial covariates of the CAR which might otherwise confound such a study (see Law et al., 2013; Stalder et al., 2016), as demonstrated by the modelling of described in 3.2.3. However, the possibility of there being unknown biobehavioural influences beyond this which may simultaneously dictate both CAR and EF cannot be entirely excluded.

Finally, it is worth note that the interpretation that a differential association between CAR and SC implies an association with structure or function of the pre-frontal cortex relies upon SC being a particularly sensitive measure of pre-frontal cortex function. A possibility remains that SC could be indicative of things other than functional integrity of this brain region, such as connectivity or, indeed, something more transient like neurotransmitter function. Further, if the CAR were to be associated with a more general cognitive resource such as attention or processing speed, this too could account for such associations if the present cognitive measures are susceptible to changes in this general resource. Evidence to date does not suggest associations with these other factors, but it is worth note that there remains much to be understood in terms of both cognition and CAR which might illuminate the nature of this relationship.

5 Conclusions

The results of the present study are in agreement with Law et al. (2015), and further provide a first demonstration of an association between the CAR and attention switching (or set shifting) performance in the afternoon of the same day, in healthy young adults. Considered alongside previous findings of CAR associations with other, similar, indices of set shifting in older adults (Evans et al., 2012), this presents a consistent body of evidence for an association between the CAR and this aspect of cognition. Such an association has potentially important implications for understanding the function of the CAR as a time-of-day marker within the circadian rhythm of cortisol secretion (Law et al., 2013; Clow et al., 2014), and thus as a potential modulator of pre-frontal cortex-associated executive functions.

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7 Tables and Figures

Table 1: Descriptive statistics on day 1 and day 2 for cortisol samples (0-45min), afternoon cortisol, sleep variables, AST reaction latency, switch cost (SC), prior day alcohol consumption, prior day exercise, and CAR measures.

	Day 1			Day 2		
	Mean	SD	N	Mean	SD	N
Cortisol S1 (0 min) (nmol/l)	8.31	5.35	40	7.55	3.73	34
Cortisol S2 (15 min) (nmol/l)	12.77	6.64	40	11.98	5.60	34
Cortisol S3 (30 min) (nmol/l)	16.71	7.70	40	16.89	8.15	34
Cortisol S4 (45 min) (nmol/l)	17.04	8.99	40	17.15	9.86.	34
Cortisol S5 (Afternoon) (nmol/l)	6.51	4.32	40	6.35	5.34	34
Time of awakening (hh:mm)	6:57	1:17	40	7:10	0:59	34
Sleep duration (hh:mm)	6:50	1:36	33	7:10	1:15	28

AST reaction latency (ms)	664.03	190.15	40	536.92	139.83	34
SC (AST switch cost inverted) (ms)	48.92	89.69	40	52.10	58.07	34
Prior day alcohol (approx. units)	0.10	0.50	39	0.06	0.35	33
Prior day exercise (approx. hrs)	0.53	1.89	38	0.26	0.83	33
MnInc (nmol/l)	7.20	6.22	40	7.80	6.11	34
AUCg (nmol/l)	42.15	18.97	40	41.22	18.89	34

MnInc = Mean Increase (0-45 min post-awakening),

AUCg = Area under the curve with respect to ground (0-45 min post-awakening).

Table 2: Pearson correlations between CAR, session, accuracy, time of awakening, and cognitive measures

Measure	1	2	3	4	5
1. CAR	-				
2. SC	.19	-			
3. Session	.05	.02	-		
4. Sampling accuracy (delay)	-.12	.05	.14	-	
5. Time of awakening	-.36**	-.22	.09	.12	-
6. AST reaction latency	-.21	-.08	-.36**	-.02	-.06

**= Correlation is significant at the .01 level.

Table 3: F-ratios, df and significances associated with parameters in mixed regression modelling of z-scored switch cost (ZSC) data.

Model Parameter	*df* (num, denom)	*F*	*p*
Intercept	1, 40.17	0.016	.901
ZCAR	1, 65.24	4.280	.043
Session	1, 33.81	0.204	.654
ZCAR*Session	1, 41.51	0.001	.979

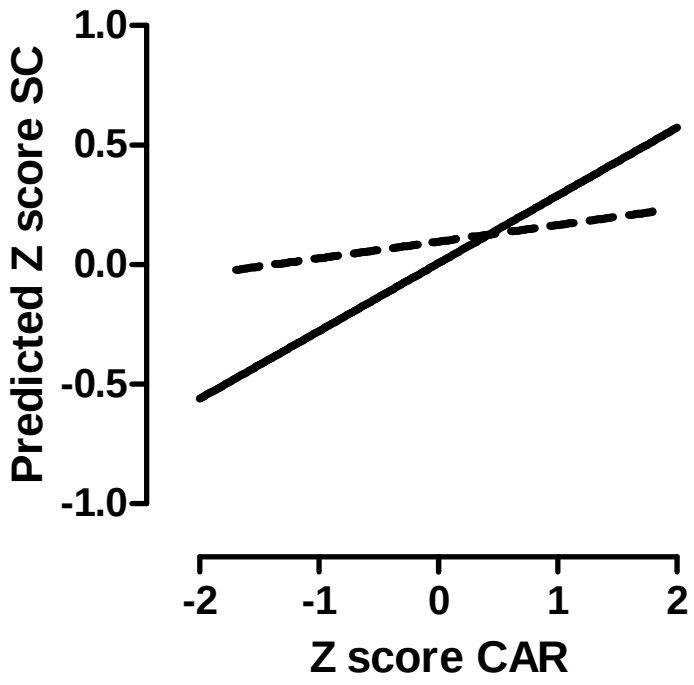

Figure 1: Line of fit equations from mixed modelling for predicted z-scored switch cost (ZSC; 'Fixed Predicted Values'; Y axis) and ZCAR mean increase (X axis) by sampling accuracy. Solid line indicates accurately timed CAR data; dashed line indicates inaccurately timed CAR data.