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https://dx.doi.org/10.1016/j.psyneuen.2017.12.016

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PII: S0306-4530(17)30432-8
DOI: https://doi.org/10.1016/j.psyneuen.2017.12.016
Reference: PNEC 3794

To appear in:

Received date: 8-5-2017
Revised date: 20-12-2017
Accepted date: 20-12-2017

Please cite this article as: Shi, Xia, Sun, Xiaofang, Yao, Zhuxi, Yuan, Yiran, Wu, Jianhui, Clow, Angela, The cortisol awakening response predicts response inhibition in the afternoon of the same day.Psychoneuroendocrinology https://doi.org/10.1016/j.psyneuen.2017.12.016

The cortisol awakening response predicts response inhibition in the afternoon of the same day

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Highlights

- A greater CAR was associated with a stronger N2.
- In CAR responders the CAR was negatively related to the false alarm rate of NoGo-trials.
- The CAR predicts response inhibition in the afternoon of the same day.

Abstract: The cortisol awakening response (CAR) is the rapid increase of cortisol levels 30–45 minutes after awakening in the morning. Numerous studies have indicated the relationship between the CAR and cognition. However, little is known about daily variation in the CAR and cognitive function in healthy adults. The aim of the present study was to investigate whether the CAR predicted the response inhibition function on the same day in both behaviour and the dynamic time course of brain processing. The saliva samples of 47 healthy men were collected at three time points: immediately on awakening, 30 minutes and 45 minutes post-awakening in the morning. Participants performed a Go/NoGo task while electroencephalograms (EEG) were recorded in the afternoon of the same day. The results showed that a greater CAR was associated with a stronger N2. In the sub-group of CAR responders (n = 33) the CAR was negatively related to the false alarm rate of NoGo-trials. Our findings suggested that the CAR was predictive of the function of response inhibition in both the earlier cognitive step (i.e., conflict monitoring) and the behavioural performance of response inhibition on the same day in healthy men.

Keywords: Cortisol awakening response; Response inhibition; Event-related potential; N2; False alarm

1. Introduction

The cortisol awakening response (CAR), first established by Pruessner et al. (1997), is the rapid increase of cortisol levels 30–45 minutes after awakening in the morning. Previous studies have suggested that the daily CAR provides energy in anticipation of upcoming demands (Adam et al., 2006; Fries et al., 2009; Hellhammer et al., 2007; Kudielka and Kirschbaum, 2003). As a kind of neuroendocrine phenomenon superimposed on the circadian rhythm of cortisol, the CAR has been related to a wide range of psychosocial and physical variables (Mangold et al., 2010; Pruessner et al., 2003).

The relationship between CAR magnitude and cognition has been investigated in several studies (Aas et al., 2011; Almela et al., 2012; Evans et al., 2011; Evans et al., 2012). Most studies treated the CAR as a trait biomarker by averaging CAR across several days and focused on patients and older adults. Some studies have found a positive correlation between the CAR and cognition. For example, Aas et al. (2011) found a blunted CAR was related to a deficit in verbal memory and processing speed in patients with first-episode psychosis. Evans et al. (2012) showed that the CAR was positively associated with executive function in older age. In contrast, other studies suggested a negative relationship between the CAR and cognition. For example, Almela et al. (2012) indicated that a greater CAR was associated with poorer declarative performance in older men and women.

Recently, the relationship between daily variation in the CAR and cognitive function in healthy adults has received growing attention. Law et al. (2015) employed a 50-day case study and found that during a day following a greater CAR the participant had better cognitive flexibility, one important aspect of executive functions. A recent study from our lab found a similar positive relationship between the CAR and brain function, that is, a greater CAR predicted higher resting-state intrinsic functional connectivity of the medial prefrontal cortex (mPFC) with the other brain areas in the afternoon of the same day (Wu et al., 2015). The results of Hodyl et al. (2015), however, suggested a negative association between the CAR and the degree of learning and the performance of memory. While Moriarty et al. (2014) found an inverted U-shaped relationship between the CAR and spatial working memory on the same day. The difference between these results could be due to the difference in cognitive functions and the methodology used, such as the time points when the cognitive tasks

were performed. There is also a gap in our knowledge as to whether the association between the CAR and cognition on the same day can be extended to other aspects of executive functions, such as response inhibition. Moreover, these previous studies generally used behavioural performance as the index of cognition but behaviour is only the final output of information processing. We asked the question of whether the CAR could predict dynamic cognition during information processing and investigated the neural mechanisms underlying the association between the CAR and behaviour on the same day.

Response inhibition is "the ability to suppress behaviours that are inappropriate, unsafe, or no longer required"(Chambers et al., 2009, p. 632). It is assumed that the prefrontal cortex (PFC) plays an important role in inhibition processes (Miller, 2001). This is a key area of the brain reportedly involved in both the target of stress, cortisol, (Herman, et al., 2005) and the regulation of the hypothalamic-pituitary-adrenal axis (Fries et al., 2009; Ostrander et al., 2003). The event-related potential (ERP) technique has high temporal resolution and could be used to investigate the dynamic steps of information processing. There are two major ERP components of response inhibition, i.e., the NoGo-N2 and the NoGo-P3. The NoGo-N2 may reflect conflict monitoring and recognition of the need for inhibition (Donkers and Boxtel, 2004; Falkenstein, 1999). The NoGo-P3 may represent response evaluation and success of response inhibition (Smith et al., 2008). Previous studies have also reported an association between cortisol and response inhibition (Shields et al., 2015). For example, one study found that acute cortisol administration enhanced inhibitory performance in healthy participants (Schlosser et al., 2013). However, the relationship between the CAR and response inhibition on the same day has not yet been studied.

The aim of the current study was to examine whether the CAR predicted the response inhibition function on the same day applying both behaviour and the dynamic time course using ERP. To avoid the confusion between baseline cortisol and the CAR, the response inhibition task, in this case the Go/NoGo, was performed in the afternoon. In the light of previous studies on the relationship between the CAR and PFC functions (Law et al., 2015; Wu et al., 2015), we hypothesised that a higher CAR would be associated with increased ability for response inhibition as shown by ERP and behaviour

results.

2. Methods

2.1. Participants

Forty-nine healthy, right-handed university students were recruited through advertisements posted at universities in Beijing. All participants were male because the CAR differs between men and women (Kudielka and Kirschbaum, 2005 ; Pruessner et al., 1997; Wright and Steptoe, 2005). Participants were excluded by the following exclusion criteria: (1) Chronic use of any psychiatric, neurological, or endocrine medication; (2) any history of psychiatric, neurological, or endocrine disorder; (3) any history of major chronic physiological disorders; (4) any history of major head injury; (5) any long-term overnight work or irregular day/night patterns; (6) any current acute inflammation; and (7) excessive consumption of alcohol (more than 2 alcoholic drinks a day) or nicotine (more than 5 cigarettes a day). All participants provided written informed consent and were paid for participation. This study was approved by the Ethics Committee of Human Experimentation of the Institute of Psychology, Chinese Academy of Sciences.

2.2. General procedures

Participants came to the laboratory on the first day and first completed the informed consent form and questionnaires including demographic questions, the Symptom Checklist 90 (SCL-90) and the Adolescent Self-Rating Life Events Check List (ASLEC) (Derogatis, et al., 1973; Liu et al., 1997). Then they were instructed on how to collect saliva samples in oral form. They were also provided with a pack containing a written version of instructions for saliva collection, MotionWatch 8 (Camntech, UK), the Salivette collection device (Sarstedt, Germany) and MEMS TrackCap containers (MEMS 6 TrackCap Monitor, Aardex Ltd. Switzerland). The MotionWatch 8 recorded sleep and activity to confirm awakening times and the MEMS TrackCap containers recorded the exact time when the participants opened the container to take the saliva Salivettes. All participants used the MEMS caps. Because there were not enough devices, we used a ‘mock strategy’ with the participants

being told that the monitoring strategies were used without the true Motionwatch for some participants (n = 20). Stalder et al. (2016) suggested that this might be one way to obtain reliable data. A standardized message sent by the experimenter in the evening of the first day reminded participants to wake up between 6:00 am and 8:00 am of the second day to balance the effect of wake time. Before going to bed in the evening, participants wore the MotionWatch on their wrist until the end of saliva collection next morning. We also instructed the participants to refrain from intense exercise during the collecting period and the night before the collecting day.

On the second experimental day, participants were asked to collect saliva samples at three time points: immediately on awakening, 30 minutes and 45 minutes after awakening in the morning. They were asked to come to the laboratory between 1:30 pm and 2:30 pm. After arrival at the laboratory，

the participants rested for half an hour in a quiet room and then prepared for electroencephalogram (EEG) recording. The EEG was collected while the participants performed tasks including the Go/NoGo. Another saliva sample was collected before the Go/NoGo task began to measure the baseline cortisol level before the task.

2.3. Go/NoGo task

One of the most common measures to investigate response inhibition is the classical Go/NoGo task. The procedures followed the protocol of previous studies performed in our laboratory (Ma et al., 2015; Wu et al., 2017; Zhang et al., 2015). The only difference in the current study is that we did not use O/X but the digit 1/9 as the stimulus. Both digits and letters have been commonly used as in Go/NoGo task in literature (Duan et al., 2009; Scholz et al., 2009). Participants viewed a series of digits with a size of 2 cm x 1 cm (either the digit 1 or 9) in the centre of a screen. All stimuli were presented on a 17-inch monitor of a Pentium processor using E-prime software, at a viewing distance of approximately 70 cm. Each stimulus was presented in white on the black screen for 150 milliseconds (ms), with randomly varying inter-stimulus intervals between 1200 ms and 1500 ms. Participants were explicitly instructed to respond as fast as possible to the appearance of the digit of Go trials by pressing a button on the keyboard with their index finger of the right hand, and to

withhold their response for the NoGo trials. The response window was 1000 ms. Response assignments between the two digits and Go/NoGo trials were counterbalanced across participants. Ten practice trials preceded two blocks of 240 trials each, of which 20% were NoGo and 80% were Go trials. After the first block, participants were allowed a rest break for approximately 1 minute. Total task duration was about 12 minutes.

2.4. Questionnaires

We measured the psychological disorder of the participants by the SCL-90. The SCL-90 is a 90-item self-reported scale, and participants were asked to refer to their feelings and behaviour during the last one week. Each item has a 5-point Likert scale (0 for not at all, 4 for extremely). We used the Chinese version, which demonstrated adequate reliability and validity (Wang et al., 1984). The Adolescent Self-Rating Life Events Check List (ASLEC) was also used to measure life events of the participants (Liu et al., 1997). The ASLEC consisted of 27 life events such as death in the family and having an accident. Participants were asked to indicate whether the event occurred in the previous 12 months. The total score is computed by summing the scores on all the items of whether the event occurred. Higher scores reflect higher levels of stress.

2.5. Salivary cortisol and analysis

Saliva samples were collected to measure salivary cortisol using a pre-labelled Salivette collection device, in which saliva was absorbed in a cotton roll. Pre-labelled Salivettes were stored in MEMS TrackCap containers in advance. The participants were asked to remove the swab from the suspended insert and chew the cotton roll for about 2 minutes. We instructed the participants take the first saliva sample immediately upon awakening without drinking water and doing anything else. Before all three saliva samples were collected after awakening, participants were not allowed to eat anything or brush their teeth to avoid saliva contamination. Although we allowed participants drink water between sampling, we asked them not to drink water 2 minutes before each sampling. After taking the first saliva sample, the participants were asked to record their awakening time in a diary and time of collection of saliva samples on the Salivette label to ensure compliance with the timing protocol. The

participants were also asked to complete a sleep log in which a record of participants' perceived sleep quality during the previous night was obtained via a 10 cm visual analogue scale where 0 cm was 'very bad' and 10 cm was 'very good'.

Saliva samples were stored at -20 °C until assayed. Saliva samples were centrifuged for 5 minutes at 3000 rpm to separate the saliva into the outer tube. The saliva concentration was determined by an electrochemiluminescence immunoassay (ECLIA, Cobas e 601, Roche Diagnostics, Mannheim, Germany). We conducted quality controls using Elecsys PreciControl Universal. None of them fell outside of the excepted range. The inter-assay CV of quality controls analysed in the current study was less than 4% which was acceptable according to Schultheiss and Stanton (2009). The intra-assay CV for low quality control and high quality control were all below 6%.

2.6. ERP recording and preprocessing

The EEG was recorded using 64 Ag/AgCl electrodes embedded in an elastic cap (Neuroscan Inc., Charlotte, NC). Electrodes were distributed according to the international 10–20 system. Reference electrodes were attached to the left mastoids and the EEG was re-referenced off-line to the average of the left and right mastoids. Signals were amplified with a 0.05 to 100 Hz band pass filter and digitization rate of 1000 Hz. A horizontal electro-oculogram (EOG) was recorded from two electrodes positioned adjacent to the outer canthus of each eye. Vertical EOG was recorded via two electrodes attached above and below the left eye. All electrode impedances were kept below 5kΩ.

The EEG data of the Go/NoGo task were digitally filtered with a 30Hz low pass filter and epochs were defined as 200 ms pre-stimulus to 600 ms post-stimulus. Segments were baseline corrected to the -200 ms period. Prior to averaging, trials with voltages above 100μV or below -100μV were rejected.

2.7. Data analysis

For the ERP data, grand average waveforms for stimulus-locked correct hits for Go trials and correct rejected NoGo trials were averaged for the N2 and P3 components. Trials with a RT below 150 ms were deleted from the average of N2 and P3 components of Go trials. The peak amplitude and

latency of the N2 and P3 components were measured at FCz and Cz, respectively, where there were maximal peak amplitudes. The behavioural performance data, the false alarm rates in the NoGo trials, was also analysed. To correct for skewed distributions, salivary cortisol values were log transformed. The absolute increase of cortisol of every participant（peak concentration minus that on awakening）was used as an index of the CAR to examine its associations with response inhibition (Law et al., 2013; Steptoe and Ussher, 2006).

Previous research has indicated that if the first saliva sample is not collected immediately after awakening, the reliability of the CAR is compromised (Thorn et al., 2006). However, even well-intentioned participants are not always able to accurately determine their awakening time. This can lead to delays in collecting the first saliva sample that are enough to cause CAR deviation. Kupper et al. (2005) used electrocardiogram (ECG) and impedance cardiogram (ICG) data and found that participants with a negative CAR showed a mean delay of 42 minutes between verified and self-reported awakening times. Accordingly, analyses were performed on the entire data set and also when divided into two groups: 1) those who had a positive CAR (i.e., CAR > 0) and 2) those who had a negative CAR (i.e., CAR < 0). This method of dichotomizing the CAR has been used in some studies (Almela et al., 2012; Hidalgo et al. 2016; O'Connor et al., 2009; Walker, et al., 2011). Although a positive CAR is no guarantee of protocol adherence, this method maximizes the proportion of accurate CARs to biased CARs (Stalder et al., 2016).

Data were analysed using the Statistical Package for the Social Sciences (SPSS 20.0). Associations between the CAR and ERP/behaviour variables were first examined using correlation analyses, because some studies have reported that a lower CAR was related to poorer sleep quality (Backhaus et al., 2004; Lasikiewicz et al., 2008) and that the pre-test cortisol level might affect the cognitive testing. The psychological disorder and life events may affect cortisol levels too (Fries et al., 2009; Pruessner et al., 2003). Hierarchical regression analyses were conducted to further investigate the relationship between the CAR and response inhibition with the CAR as an independent variable and the ERP data and behavioural performance data as a dependent variable while SCL-90, life events, pre-task cortisol and sleep quality were entered as covariates.

3. Results

3.1. Descriptive data

One participant who went back to sleep after awakening was excluded. Another participant was excluded because the number of acceptable EEG trials for NoGo condition was less than ten and did not achieve the number of acceptable trials. The final sample (n = 47) ranged in age from 18 to 26 years (M = 22.44, SD = 1.92).

First, we compared the difference in the CAR between the mock-strategy group (n = 20) and the actual monitored -strategy group (n = 27), no difference in the CAR was found between these two groups (t(45) = 0.859, p = 0.395; mock-strategy group: mean = 0.107 nmol/L, SD = 0.157; actual monitored-strategy group: mean = 0.068 nmol/L, SD = 0.150). Of the total 47 samples, 70.2% of the participants showed a positive CAR (n = 33) and 29.8% of the participants showed a negative CAR (n = 14) (Figure 1). The number of nonresponders in the actual monitored -strategy group was 10 and the number of nonresponders in the mock-strategy group was 4. A *t*-test analysis between these two subgroups revealed that the positive CAR group had lower first cortisol concentrations than the negative CAR group (t(45) = 2.583, p = 0.013).

In order to test for the potential effects of participant non-adherence to the protocol, the MEMS times for Sample 1 were compared with the self-reported awakening time written in the log by the participants. There was no significant difference between these two groups regarding the delay between the MEMS times and self-reported awakening times (t(45) = -1.113, p = 0.271; CAR > 0 group: Mean = 0.03 minutes, SD = 2.27 ; CAR < 0 group: Mean = -0.71 minutes, SD = 1.59). Moreover, we compared the MEMS times with the awakening times recorded by the MotionWatch. There was also no significant difference between the two groups regarding the delay between the MEMS times for sample 1 and MotionWatch recorded awakening times (t(25) = -0.868 , p = 0.408; CAR > 0 group (n = 17): mean = 1.47 minutes, SD = 2.24; CAR < 0 group (n = 10): mean = 7 minutes, SD = 20.07).

Figure 1 here

Figure 1. CAR profiles of the positive CAR (n=33) and negative CAR (n=14) subgroups. Depicted values are means and error bars represent the SEM. To aid understanding of the figure, the values are raw values and not log-transformed values.

For the ERP data of the total sample, there was a significant difference between the two trial types in peak amplitude of the N2 and P3 components (Figure 2), with more negative N2 ($t(46) = -8.66$, $p < 0.001$) and more positive P3 ($t(46) = 9.707$, $p < 0.001$) for NoGo stimuli than for Go stimuli. For the behavioural data of the total sample, the false alarm rate on NoGo trials was 13.93% ± 9.61%. The Kolmogorov-Smirnov test showed that the variable was normally distributed (K-S Z = 0.893, P = 0.403). For the total sample, the total score of SCL-90 was 26.63 ± 33.15. The number of life events was 8.06 ± 6.39. The mean sleep quality was 6.67 cm with a standard deviation of 1.96. The mean pre-task cortisol level was 8.91 nmol/L with a standard deviation of 3.47.

Figure 2 here

Figure 2. Grand average of ERP for Go and NoGo trials. The scalp distributions are time-locked to the peak amplitude of the NoGo-N2 at FCz and NoGo-P3 at Cz.

3.2. Relationship between CAR and N2/P3

Bivariate Pearson correlation analysis indicated that the CAR was inversely associated with the peak amplitude of NoGo-N2 for the total sample ($r = -0.381$, $p < 0.01$). Note that because the N2 is a negative waveform, the observed negative correlation indicates that a greater CAR is associated with a stronger N2. But there was not significant correlation between the CAR and NoGo-N2 latency ($r = 0.114$, $p > 0.05$). Furthermore, no significant correlation was found between the CAR and the amplitude and latency of the NoGo-P3 (amplitude: $r = 0.046$, $p > 0.05$; latency: $r = -0.034$, $p > 0.05$). Results for the positive CAR group still showed a significant negative linear relationship between the

CAR and peak amplitude of NoGo-N2 ($r = -0.349$, $p < 0.05$) (for scatterplots, see Figure 3, Left) but not the NoGo-N2 latency ($r = 0.166$, $p > 0.05$) and the amplitude and latency of the NoGo-P3 (amplitude: $r = 0.078$, $p > 0.05$; latency: $r = -0.087$, $p > 0.05$).

In order to avoid confounding factors that could interfere with the CAR and response inhibition, we performed a hierarchical regression analysis with the CAR as an independent variable and the peak amplitude of NoGo-N2 as a dependent variable, while controlling for total score of SCL-90, the total number of life events, sleep quality and pre-test cortisol level. In step1, we included control variables (total score of SCL-90, the total number of life events, sleep quality and pre-test cortisol level). In step 2, we included the CAR to investigate the relationship between the CAR and ERP measures of response inhibition. The results showed that there was a significant negative linear relationship between the CAR and the peak amplitude of NoGo-N2 for the total sample ($\beta = -0.357$, $p < 0.05$). When only the samples from participants who showed a positive CAR were assessed ($n = 33$), a similar result was observed ($\beta = -0.352$, $p < 0.05$). With regard to the results with latency of NoGo-N2 and latency and amplitude of NoGo-P3, we failed to find a significant relationship with the CAR for both total sample and the positive CAR group ($\beta = -0.019$ - 0.207, $ps > 0.05$). Table 1 summarizes the main results of the regression analyses for the complete sample and for the positive CAR group.

Table 1

Regression analysis (with covariates) with CAR as predictor and NoGo-N2 amplitude as dependent variable

			β	t	p	R^2	ΔR^2
Total sample	Model 1	SCL-90	-0.334	-2.086	0.043	0.114	0.114
		Life events	0.136	0.869	0.39		
		Sleep quality	-0.154	-1.025	0.311		
(n=47)		Pre-task cortisol	0.094	0.627	0.534		
	Model 2	SCL-90	-0.282	-1.865	0.069	0.238	0.124
		Life events	0.135	0.916	0.365		
		Sleep quality	-0.183	-1.294	0.203		
		Pre-task cortisol	0.085	0.604	0.549		
		CAR	-0.357	-2.576	0.014		
CAR>0	Model 1	SCL-90	-0.383	-1.941	0.062	0.145	0.145

		Life events	0.208	1.039	0.308		
		Sleep quality	-0.256	-1.361	0.184		
(n=33)		Pre-task cortisol	0.078	0.441	0.663		
	Model 2	SCL-90	-0.361	-1.935	0.064	0.265	0.12
		Life events	0.144	0.752	0.459		
		Sleep quality	-0.272	-1.528	0.138		
		Pre-task cortisol	0.086	0.514	0.611		
		CAR	-0.352	-2.098	0.045		

Note: CAR: Cortisol awakening response

3.3. Relationship between CAR and behaviour

For the total sample, bivariate Pearson correlation analysis showed that there was no significant correlation between CAR and the false alarm rate of NoGo trials ($r = 0.019$, $p > 0.05$) . To control for the effect of potential non-adherence to the protocol, we repeated all the analyses in a sub-group of participants who showed a positive CAR. Bivariate Pearson correlation showed that CAR was negatively correlated with the false alarm rate of NoGo trials ($r = –0.372$, $p < 0.05$) for the positive sub-group (for scatterplots of this bivariate correlation, see Figure 3, right).

Figure 3 here

Figure 3. Left: Scatter plots of bivariate Pearson correlation between CAR and NoGo-N2 amplitude (Note that the N2 is a negative component, thus the observed negative correlation indicates that a greater CAR is associated with a stronger N2) (n = 33). Right: Scatter plots of the bivariate Pearson correlation between CAR and NoGo false alarm rate (n = 33).

In addition, a scatterplot showed that the relationship between the CAR and the false alarm rate of NoGo trials might be curvilinear for the complete sample (see Figure 4). The regression analyses were also carried to examine the linear and curvilinear relationships between the CAR and the false alarm rate of NoGo trials while controlling for total score of SCL-90, the total number of life events, sleep quality and pre-test cortisol level. In step 1, we included the control variables (SCL-90, life events,

sleep quality and pre-task cortisol). In step 2, we included the CAR to investigate the linear relationship between the CAR and the behavioural measures of response inhibition. In step 3, the square of the CAR was included to examine the curvilinear relationship between the CAR and the behavioural measures of response inhibition. The results showed that the CAR had a significantly negative linear association with the false alarm rate of NoGo trials in the positive CAR group (β = -0.404, $p < 0.05$) but not in the full sample (β = -0.004, $p > 0.05$) For the complete sample, the marginally significant curvilinear relationship was found between the CAR and the false alarm rate of NoGo trials (β = -0.354, p = 0.063) (Table 2).

Figure 4 here

Figure 4. Scatterplot illustrating the relationship between the CAR and the false alarm rate of NoGo trials may be curvilinear for the complete sample.

Table 2

Regression analysis (with covariates) with CAR as predictor and false alarm rate of NoGo trials as dependent variable

			β	t	p	R^2	ΔR^2
Total sample	Model 1	SCL-90	0.053	0.340	0.736	0.147	0.147
		Life events	-0.026	-0.166	0.869		
		Sleep quality	0.062	0.423	0.674		
(n=47)		Pre-task cortisol	-0.381	-2.604	0.013		
	Model 2	SCL-90	0.054	0.337	0.738	0.147	0
		Life events	-0.026	0.164	0.870		
		Sleep quality	0.062	0.415	0.681		
		Pre-task cortisol	-0.381	-2.573	0.014		
		CAR	-0.004	-0.029	0.977		
	Model 3	SCL-90	0.047	0.305	0.762	0.218	0.071
		Life events	-0.098	-0.629	0.533		
		Sleep quality	0.052	0.359	0.722		
		Pre-task cortisol	-0.371	-2.581	0.014		
		CAR	0.219	1.190	0.241		

		CAR^2	-0.354	-1.909	0.063		
CAR>0	Model 1	SCL-90	0.060	0.309	0.760	0.178	0.178
		Life events	-0.153	-0.780	0.442		
		Sleep quality	0.047	0.252	0.803		
(n=33)		Pre-task cortisol	-0.400	-2.295	0.029		
	Model 2	SCL-90	0.085	0.487	0.636	0.336	0.158
		Life events	-0.226	-1.245	0.224		
		Sleep quality	0.028	0.168	0.867		
		Pre-task cortisol	-0.391	-2.449	0.021		
		CAR	-0.404	-2.532	0.017		
	Model 3	SCL-90	0.090	0.499	0.622	0.339	0.003
		Life events	-0.209	-1.100	0.282		
		Sleep quality	0.026	0.148	0.883		
		Pre-task cortisol	-0.400	-2.436	0.022		
		CAR	-0.594	-1.072	0.293		
		CAR^2	0.202	0.358	0.723		

Note: CAR: Cortisol awakening response; CAR^2: Square of the cortisol awakening response.

4. Discussion

The present study examined the relationship between the CAR and response inhibition in healthy men on the same day. A greater CAR is associated with a stronger N2 for both the complete sample and the positive CAR sub-group. Moreover, for those participants who showed a positive CAR, CAR was negatively related to the false alarm rate of NoGo-trials. It is important to note that the relationships were still significant after controlling for the total score of SCL-90, the total number of life events, pre-task cortisol level and sleep quality.

According to preliminary analyses of adherence to the salivary sampling protocol, 70.2% of the participants showed a positive CAR while 29.8% of the participants showed a negative CAR. This proportion is similar to previous studies (Hidalgo et al., 2016; Hodyl et al., 2015), although we used electronic monitoring devices for most participants in our study and there was no adequate objective evidence indicating that the participants in the negative CAR subgroup were non-adherent. However, the data of the MotionWatch were based on the participant's physical activity and might be not accurate if the participants had woken up but they did not move (i.e., sleep inertia effects) (Balkin et al., 2002). Moreover, the results showed that the negative CAR group had significantly higher first cortisol concentrations than the positive CAR group, which led us to speculate that the first collection

time might be delayed after the actual awakening time of the negative group.

Our ERP results found that a greater CAR is associated with a stronger N2 for both the complete sample and the positive CAR sub-group，predicting 12.0% of its variance in individuals with a positive CAR and 12.4% in full sample. According to the literature, the NoGo-N2 has been linked to the earlier step of response inhibition, that is, the process of conflict monitoring or detection (Falkenstein, 2006), and the larger amplitude of N2 suggested that there was an increased intensity of neural monitoring to prevent response execution or to prepare for response inhibition. The results of the present study suggest that the higher CAR levels predict greater neural activity associated with conflict monitoring and detection during the response-inhibition process. Support for the CAR being associated with brain function on the same day has been shown in some studies. For example, Clow et al. (2014) found that an increased CAR corresponded to increased same-day neuroplasticity in healthy adults. The current result is also similar to that of our previous study (Wu et al., 2015), which found that total cortisol secretion over the first hour after awakening (another aspect of CAR) predicts a stronger intrinsic functional connectivity of the mPFC in the afternoon of the same day. However, our previous study measured resting-state brain activity without any task and behavioural data. Our current study extends this study by suggesting that the CAR predicts response inhibition function in both behavioural performance and neural activity related to the function of the response inhibition.

Our behavioural results also revealed that the CAR was negatively associated with the false alarm rate of the Go/NoGo task，predicting 15.8% of its variance in individuals with a positive CAR. As a decreased false alarm rate in the Go/NoGo task is considered a better function in response inhibition, the current results suggested that a higher CAR was associated with increased inhibitory control capabilities for the positive CAR sub-group on the same day. This finding is consistent with a previous study showing that greater CAR predicted better cognitive flexibility on the same day (Law et al., 2015). Our behavioural data demonstrate that CAR is also positively associated with the ability of another important executive function, that is response inhibition, in healthy men. Notably, the cognitive task was performed immediately after the CAR acquisition in Law and his colleagues' study (2015). In the current study, we delayed the cognitive task until the afternoon to prolong the time

interval between the CAR and the cognitive task to several hours, while controlling the effect of baseline cortisol immediately before cognitive tasks.

However, we failed to find significant linear relationship between the CAR and the false alarm rate of the Go/NoGo task for the total group. An inverted-U quadratic trend was found between CAR and the false alarm rate of the Go/NoGo task for the total sample. A previous study also suggested that the CAR was linearly related to declarative memory performance in the older participants who showed the positive CAR and this relationship was quadratic (i.e., inverted U-shaped form) for the total sample (Almela et al., 2012). Among the total group, those who showed the negative CAR may did not collect the first salvia sample once awakening, but some time later (Thorn et al., 2006). According to Almela et al.(2012), for those who showed a negative CAR, "their samples showed the recovery after this increase had already happened" and therefore have a relative large cortisol decrease. It seems likely that "those who showed a relative large cortisol decrease had a relative large CAR (thus making a large decrease possible)". This reasoning can explain the left side of the inverted U relationship (see Fig. 4), as those with a large cortisol decrease (probably large CAR) had a less false alarm rate (thus better response inhibition performance) just as those participants on the right side of the inverted U curve (i.e., those who have a positive CAR).

We emphasize here that no causality could be attributed to the association between the CAR and response inhibition observed in the current study. There are two possible interpretations that may explain these results. Firstly, the CAR may enhance PFC function, which is consistent with the assumption that the CAR functions as a provider of neuroendocrine energy for the behaviour of the upcoming day (Adam et al., 2006; Fries et al., 2009; Hellhammer et al., 2007; Kudielka and Kirschbaum, 2003). This promotion could be achieved by a slow effect of cortisol. Previous research has indicated that the corticosteroids might exert both rapid-acting, nongenomic and slow, genomic effects to serve brain functions (Henckens et al., 2011). Furthermore, these rapid and slow effects of corticosteroids have been shown to have different consequences in cognitive function. For example, results revealed that acute stress inhibited working memory (Qin et al., 2009). The slow effects of corticosteroid administration, however, improved working memory (Henckens et al., 2011; for a meta analysis, see Shields et al., 2015). As a kind of naturally secreted cortisol, CAR may enhance another

important PFC function, the response inhibition, by the slow genomic effect. However, we should be cautious about the direction of the association. Contrary to our results, in the meta-analysis conducted by Shields et al. (2015), they found that the slow, genomic effects of cortisol administration have a trend of impairing response inhibition, although this effect did not achieve statistical significnce ($p>0.05$).

The second possible explanation for these results is that the PFC, which mediates the function of response inhibition, may also play a role in maintaining homeostasis in the morning after awakening, through the dynamics of the CAR. Although the specific role of the PFC in the regulation of the CAR is unknown, the hypothalamic-pituitary-adrenal axis activity in general is regulated by distinct brain regions including the PFC (Herman et al., 2005). Some studies have assumed that the PFC might be also involved in regulating the CAR (Boehringer et al., 2015; Fries et al., 2009; Frokjaer et al., 2013).

Some limitations of our study have to be considered. First of all, our study only investigated male participants. The CAR has been shown to be different in men and women (Kudielka and Kirschbaum, 2005； Pruessner et al., 1997; Wright and Steptoe, 2005). Thus the association between CAR and the response inhibition observed in this study might not be generalized to women. Secondly, although we have used electronic devices to monitor awakening time, we still do not know the exact awakening time of the participants due to the sleep inertia phenomenon and the fact that electronic devices (such as MotionWatch) depend on the physical activity of the participants. Thirdly, we assayed the samples in singlicate, which does not allow us to assess intra-assay %CV for all samples and may entail greater measurement error for each sample. We will run samples in duplicate for our later studies. However, the Elecsys PreciControls were run in duplicate in the same plates alongside the study samples and their intra-assay CVs were all less than 6%, which may infer that the intra-assay %CV of our own sample was acceptable (Schultheiss and Stanton, 2009). Finally, our study only examined the response inhibition ability, more studies are needed to test whether the findings in the current study can be extended to other cognitive functions on the same day.

In conclusion, the results of the present study suggest that CAR is predictive of the function of response inhibition in both ERP and behavioural performance on the afternoon of the same day in

healthy men. We have shown that with greater CAR, there is greater cognitive neural activity during the earlier monitoring step of the response inhibition and fewer false alarms in the NoGo trials.

Contributors

Author S.X. collected, analysed the data and wrote the manuscript. Author W.J.H. designed the study and revised the manuscript. Authors S.X.F., Y.Z.X and Y.Y.R. participated in the design of the study and the acquisition of the data. Author A.C. participated in the interpretation of the data and the revision of the manuscript. All the authors approved the final manuscript.

Conflict of interest

The authors report no conflicts of interests.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (31771246, 31530031, 31600924) and the Natural Science Foundation of SZU (000174).

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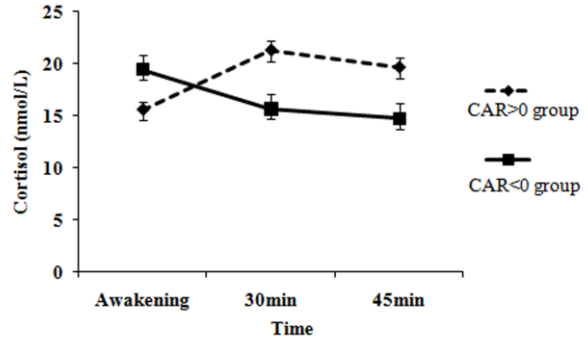

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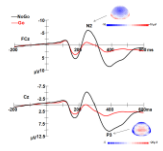

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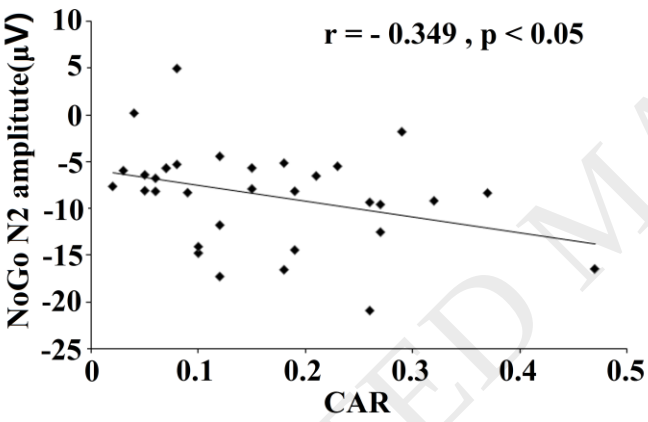

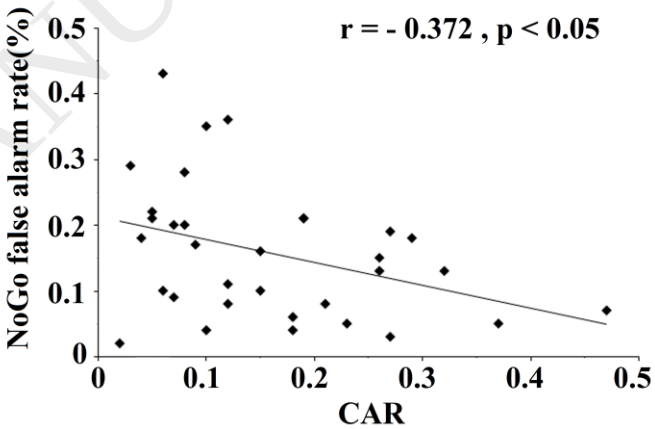

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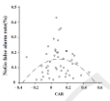

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