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The Awakening Cortisol Response: Methodological Issues and Significance

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The awakening cortisol response (ACR) is a discrete and distinctive part of the cortisol circadian cycle. In healthy adults salivary free cortisol concentrations increase by between 50 and 160% in the first 30 min immediately post-awakening (approximate average increase of 9 nmol/l, range 4–15 nmol/l, estimated to be equivalent to about three secretory episodes). However there are no agreed norms for the absolute concentrations of free cortisol in saliva either immediately post-awakening (range of 4.7–18.5 nmol/l) or 30 min post-awakening (range of 8.6–21.9 nmol/l). This review explores reasons for these discrepancies in normative data including confounding factors such as gender, age, awakening time, light and participant adherence. Although the physiological role of the ACR has not been clearly defined evidence is discussed that suggests it is under a distinct regulatory influence, different from the rest of the diurnal cortisol secretory cycle. Despite the difficulties associated with its measurement a range of studies have demonstrated an association between the ACR and psychosocial variables, stress and health. However it remains unclear whether positive affect and good health are consistently associated with larger or smaller awakening responses. It is early days in the search for the role and significance of the ACR. Its putative role in the regulation of physiological function across the day (e.g. the immune system) and its sensitivity to psychosocial variables make it a prime candidate as an intermediary linking mind and health.

Keywords: Adrenal cortex; Circadian rhythm; Confounding factors; Health; Light; Participant adherence

INTRODUCTION

Cortisol secretory activity is characterised by peak levels following awakening and a declining pattern thereafter (e.g. Edwards *et al.*, 2001a). Dysregulation in this diurnal rhythm is associated with various pathologies, both psychological and somatic. For example, abnormal profiles are evident in individuals experiencing major depression (Trestman *et al.*, 1995; Goodyer *et al.*, 1996; Yehuda *et al.*, 1996; Deuschle *et al.*, 1997b; Weber *et al.*, 2000; den Hartog *et al.*, 2003) and in cancer patients (Touitou *et al.*, 1996; Mormont *et al.*, 1998; Sephton *et al.*, 2000). The subject of this review is a discrete aspect of that circadian cycle: the awakening cortisol response (ACR). The ACR (which can be defined as the period of cortisol secretory activity in the first 45–60 min immediately post-awakening) has also been associated with a wide range of psychosocial variables, stress and health and may play a significant role in the regulatory balance of the immune system (see later). However, the ACR literature is relatively new, there are inconsistencies and few observations have been clearly replicated. The dynamic nature of the ACR makes it both fascinating to study and likely to be of physiological significance but these same characteristics make it difficult to accurately quantify. Various experimental protocols and approaches to statistical analyses have been used, which can make interpretation of the literature problematic. It is the aim of this review to explore some of the methodological issues surrounding this measure and to review its significance for health.

METHODOLOGICAL ISSUES

Stability of the Awakening Response Across Days

The ACR was first brought to the notice of researchers in 1995 when Pruessner and co-workers reported that in

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healthy participants there was a 50–100% increase in the concentration of free cortisol as measured in saliva (known to correlate highly with physiologically active "free" cortisol concentrations in the blood, Kirschbaum and Hellhammer, 1989) within 30–45 min of awakening. Correlational analysis revealed the response to be reasonably stable for individuals across days: for the first awakening sample (rho = 0.53, $p = 0.001$), and for the 20-min sample (rho = 0.71, $p = 0.001$). In addition the increase in cortisol concentration from immediately to 20 min post-awakening was relatively consistent across two consecutive days (rho = 0.43, $p = 0.002$). Reliability of the area under the cortisol curve (with reference to zero i.e. an estimate of the total amount of cortisol secretory activity) for the first 45 min after awakening, has also been demonstrated (correlations range from 0.39 to 0.67 across consecutive days (Pruessner *et al.*, 1997; Wust *et al.*, 2000b; Edwards *et al.*, 2001a)).

In its quantification the ACR is problematic. A distinction can be drawn between the best estimate of total cortisol secretory activity during the post-awakening period (such as the area under the cortisol curve) and various measures of the dynamic of the change in concentration from awakening to peak levels (typically 30 min post-awakening). Issues relevant to its measurement are discussed in a following section but for the purposes of this review the term ACR describes this period of cortisol secretory activity as a whole and makes no reference to any specific aspect of its measurement.

Normative Values in Healthy Adult Populations

The ACR has been clearly and consistently demonstrated in all published studies that touch upon this aspect of neuroendocrine activity. However the absolute values of salivary free cortisol concentration at comparable post-awakening time intervals vary widely from study to study. Table I shows the approximate values (derived from the manuscripts) in the 12 papers that provide data for healthy adults (Fig. 1).

It is of concern that so much variation exists in the published norms for this variable. Undoubtedly, one of the reasons that such discrepancies occur is that the ACR is sensitive to a range of confounding factors (see below). It is also probable that different hormone assay systems contribute to the variations between published data—an issue that has been explored in a recent comparison of cortisol determination in saliva (Garde *et al.*, 2003). However, despite the wide range in absolute values obtained the reported change in cortisol concentration from awakening till 30 min post-awakening in healthy adults is relatively consistent between studies at 9.3 ± 3.1 nmol/l (see Table I). This increase may represent the cumulative effect of as many as three cortisol secretory episodes during this brief post-awakening period, where one minimum secretory episode is taken as an increase in salivary free cortisol concentration of 2.5 nmol/l (see Wust *et al.*, 2000b).

TABLE I The ACR in healthy adult participants, in the absence of any experimental manipulation

		Post-awakening					From 0 to 30 min PA	
Authors	Participants	0 min	15 min	30 min	45 min	60 min	Increase (%)	Change (nmol/l)
Brooke-Wavell *et al.* (2002)	Healthy, $n = 36$, all f, 19–45 y	4.7		8.6			83	3.9
Edwards *et al.* (2001a)	Healthy, $n = 42$, 32f, 10m, 23–59 y, mean 35 y	7.3	11.9	17.6	12.3		141	10.3
Hucklebridge *et al.* (1998a)	Healthy, $n = 30$, 21f, 9m, 20–45 y, mean 25.8 y	9.0		23.0			156	14.0
Hucklebridge *et al.* (1998b)	Healthy, $n = 41$, 30f, 11m, 20–40 y, mean24 y	10.0		25.0			150	15.0
Hucklebridge *et al.* (1999)*	Healthy, $n = 30$, 14m, 13f, 20–66 y, mean 30 y	10		19.5			95	9.5
Hucklebridge *et al.* (2002)*	Healthy, $n = 16$, 8m, 8f, 20–37 y, mean 24.3 y	5.5	9.5	13.0	11.0		136	7.5
Kudielka and Kirschbaum (2003)*	Healthy, $n = 105$, 53f, 52m, 4–75 y (8 children)	18.5	24.5	28.0	27.5	24.5	51	9.5
Kunz-Ebrecht *et al.* (in press)*	Healthy, $n = 227$, 69m, 59f, 45–59 y	17.7		29.1			64	11.4
Pruessner *et al.* (1997)*	Healthy, $n = 70$, 35m, 35f age 19–37 y, mean 26.5 y	14.0	16.5	22.0	23.0	21.0	57	8.0
Pruessner *et al.* (1997)*	Healthy $n = 40$, 20m, 20f age 59–82 y, mean 70.4 y	16.0	21.0	24.0		22.0	50	8.0
Schmidt-Reinwald *et al.* (1999)*	Healthy, $n = 22$, 15f, 7m, mean 22.4 y	11.0	14.5	17.7	17.0	18.5	61	6.7
Wust *et al.* (2000b)*	Healthy, $n = 509$, 319f, 190m, 18–71 y, mean 37.3 y	15.0		23	22.5	20.5	53	8.0
Mean, nmol/l ± (SD between studies); n = number of studies†		11.6 (4.6) $n = 12$	16.3 (5.6) $n = 6$	20.0 (5.9) $n = 12$	18.9 (6.5) $n = 6$	21.3 (2.2) $n = 5$	91.4 (42.4) $n = 12$	9.3 (3.1)

* Approximate values derived from published illustrations.
† Each study was equally weighted regardless of number of participants.
Approximate mean salivary free cortisol concentrations (nmol/l) in the first hour after awakening, derived from published values*. The final columns show the approximate percentage increase and change in cortisol concentration (nmol/l) from 0 min post-awakening to 30 min post-awakening. The studies are listed in alphabetical order by first author. f, female; m, male; y, years; min, minutes; PA, post-awakening.

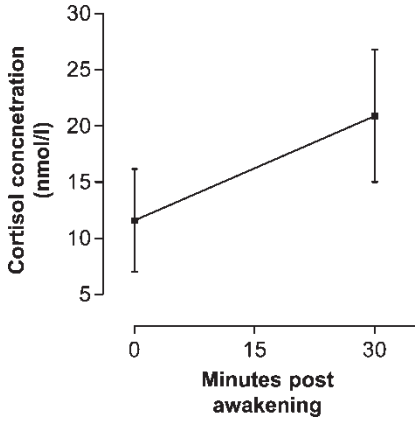

FIGURE 1 The ACR in healthy adult participants: a composite from the 12 studies (detailed in Table I) with values at awakening and 30 min post-awakening (study means ± SD between studies, of salivary free cortisol concentration in nmol/l). Values are significantly greater at 30 than at 0 min ($t = 4.028$, df $= 22$, $p < 0.001$).

Confounding Factors

Gender Issues

Premenopausal females were reported to have a more sustained ACR, remaining higher than matched males at 30, 45 and 60 min post-awakening (Pruessner *et al.*, 1997; 1999; Wust *et al.*, 2000b), but this has not been replicated (Edwards *et al.*, 2001a; Kudielka and Kirschbaum, 2003) although recently it has been reported that females have a greater ACR on weekdays but not at weekends (Kunz-Ebrecht *et al.*, in press). Females on oral contraceptives were found to have an attenuated ACR (Pruessner *et al.*, 1997; 1999). The ACR is reported not to be associated with phase of the menstrual cycle (Kudielka and Kirschbaum, 2003).

Age

The ACR was reported to be unrelated to age in adults (Pruessner *et al.*, 1997; Wust *et al.*, 2000b; Edwards *et al.*, 2001a) or adolescents (Ellenbogen *et al.*, in press). The early study of Pruessner *et al.* (1997) included a small population of children, under ten years of age, who demonstrated very little evidence of an ACR, though this is not commented upon by the authors and might easily represent a sampling artefact. The possibility that the ACR matures during childhood remains uninvestigated. Recent studies have provided evidence of a negative relationship between the ACR and advancing years. It has been observed that increasing age (up to 75 years) is associated with a greater cortisol concentration in the first waking samples but a muted response thereafter (Kudielka and Kirschbaum, 2003).

Smokers

Initially smoking (smokers vs. non-smokers) was reported not to be associated with the ACR (Pruessner *et al.*, 1997; Edwards *et al.*, 2001a). A slightly attenuated ACR for smokers was found in one study (Wust *et al.*, 2000b) and more recently the ACR is reported to be greater in smokers compared to non-smokers (Kunz-Ebrecht *et al.*, in press).

Awakening Time

There has been controversy about the association between time of awakening and the ACR. Frequently reported not to be associated with awakening time (Pruessner *et al.*, 1997; Wust *et al.*, 2000b; Brooke-Wavell *et al.*, 2002; Kunz-Ebrecht *et al.*, in press) moderately strong relationships have been reported in three studies such that the ACR was larger in early risers (Edwards *et al.*, 2001b; Kudielka and Kirschbaum, 2003; Federenko *et al.*, in press). It is possible that the association between waking time and the ACR can be obscured by age. Early waking is associated with a heightened ACR but increasing age, which is associated with early waking, is also associated with an attenuated ACR (see Kudielka and Kirschbaum, 2003). Evidence is sufficient to indicate that awakening time should be documented and excluded as a possible confounding variable in all future studies. The physiological significance of the relationship between awakening time and the ACR will be explored fully in the following sections.

Effect of Light

The ACR can be modulated by light exposure following awakening, providing evidence for possible regulatory mechanisms (see later). The ACR is apparent when participants are maintained in total darkness following awakening, but is significantly enhanced by 800 lux light exposure over the same time period (Scheer and Buijs, 1999). Indeed, exposure to simulated dawn, before awakening, has been shown to heighten the ACR (Thorn *et al.*, in press). It is possible that some of the discrepancies in the literature can be attributed to variations in light exposure pre- and post-awakening. It remains possible the ACR may be affected by seasonal variations in light—a credible confounding variable yet to be investigated.

Weekday vs. Weekend Collections

Although the ACR is known to have a moderate stability across consecutive days a greater response has been reported on weekdays, which subjects rated as less happy and more stressful, compared to weekend days in the same participants (Kunz-Ebrecht *et al.*, in press). This is an important observation as it means that the ACR is sensitive to the anticipation of a potentially stressful day, and this could confound associations with other variables. Indeed not all papers indicate whether sampling days are weekend or weekday—or even a mixture of both. All these factors require documentation in order to evaluate the association of the ACR with other variables.

Participant Adherence

Without doubt the use of saliva sampling to accurately determine the physiologically active free component of cortisol (see Kirschbaum and Hellhammer, 1989) has facilitated research into the ACR. However these studies are

usually carried out within the domestic setting for which the participants, following careful verbal direction, take away a pack of written instructions and saliva sampling devices. This type of procedure relies very heavily upon participant adherence to the protocol. Recently concern has been voiced about the reliability of this approach. The ACR is especially sensitive to deviation from instructions, leading to timing errors, as the rate of increase in cortisol is often more than 100% within just 30 min, so even small deviations from the protocol would have substantial consequences for the values obtained; such small deviations in adherence are far less problematic at the end of the day when changes in cortisol concentrations are less marked.

One recent study utilised an electronic devise to track when participants removed a lid to access a cotton swab for saliva sampling to investigate adherence to protocol in a community dwelling sample of 47 participants (Kudielka *et al.*, 2003). Overall 74% of the participants correctly followed the sampling regime, taking six samples across a day, and 26% failed to take at least one sample as instructed. In particular the authors noted that 55% of the non-compliant participants failed to take the saliva sample correctly 30 min after awakening. They also reported that participants not informed that they were being monitored were significantly less compliant than the informed group and that for the non-informed group self-reported adherence to protocol was less reliable. In a further study adherence to protocol in participants unaware they were being monitored was 71%, with no difference between weekdays and weekends, although repeated sampling over a series of 7 days led to a substantial reduction in adherence in the final 3 days (Broderick *et al.*, in press). There was a statistically significant difference in the cortisol profiles of adherent vs. non-adherent participants in both of these studies, with the non-adherent group having "apparently" lower morning cortisol concentrations. Obviously this does mean that care must be taken when instructing participants to ensure they fully understand the collection protocol and how important it is to adhere strictly with the instructions. One must also build into the experimental design some method to identify non-adherent sampling. One method for doing this is the use of electronic monitoring as described, but electronic monitoring only reveals apparent times of sampling and its concordance with self-reported time of sampling, moreover this technology can give no indication to the experimenter as to the timing accuracy of the first post-awakening sample, only that the following samples are taken at the correct subsequent intervals.

In an attempt to address this issue we have recently introduced a system of probing for participant possible non-adherence. In this protocol participants are instructed to collect saliva samples according to identical regimes on at least two consecutive days (either weekdays or weekend days, but not a mixture). This enables examination of the consistency of the ACR for each individual across the two days. In one recent study (Thorn *et al.*, 2003) on young healthy participants we found that 21 out of 48 (43%) failed to show greater cortisol concentrations 15 min post-awakening (i.e. a typical response) on at least one of the study days. This group was significantly younger than the "typical" responders and there was no difference in any other psychosocial or health variable. This led us to consider that, at least in our population, the atypical cortisol profiles were more likely a result of non-adherence than to merit physiological explanation. It is desirable that future studies into the ACR adopt a method to maximise participant adherence and that profiles consequent upon non-adherence do not confuse the literature. Nonetheless, caution should be taken to examine the psychosocial and health characteristics of any group deemed to be "non-adherent" by this method as it remains possible that inconsistency in the ACR across days may, in some individuals, be a physiological feature of their ACR and not a result of non-adherence.

Factors Reported Not to be Associated with ACR

The ACR has been shown to be independent of the following factors, without published contradiction: quality of sleep, body mass index, alcohol consumption, hormone replacement therapy (Kunz-Ebrecht *et al.*, in press); sleep duration (Federenko *et al.*, in press); spontaneous awakening vs. alarm waking (Pruessner *et al.*, 1997; Wust *et al.*, 2000b); the postural shift, from supine to standing, (Hucklebridge *et al.*, 2002); blood glucose levels (Hucklebridge *et al.*, 1999); disrupted sleep (Hucklebridge *et al.*, 2000).

Detailed Methodological Considerations

When instructing participants to collect saliva samples in a domestic setting it is essential that, in addition to following the correct timing protocol, participants adhere to the following well-known methodological details: nil by mouth, other than water, for at least 30 min prior to collection; absence from smoking for at least 30 min prior to collection; no cleaning of teeth (to avoid abrasion and vascular leakage into the sample) for at least 30 min prior to collection.

Measurement of the ACR

The ACR, as measured in saliva, comprises two elements: the estimate of overall cortisol secretory activity measured in the period after awakening and the dynamic of the response (change in concentration from awakening to peak levels). Overall or total cortisol secretory activity can be quantified when three or more measures are available by calculation of the area under the curve (AUC) relative to zero[†] (or ground: AUC_G) (see Pruessner *et al.*, 2003a, for discussion of this calculation). Alternatively the increased amount secreted following awakening, using the first awakening sample gives an indication of

[†]AUC relative to zero = sample 1 + s2 + s3 + ((s4 − s1)/2).

the response: AURC[‡]—area under the response curve (see Edwards *et al.*, 2001b) or AUC_1 area under the curve with respect to increase (see Pruessner *et al.*, 2003a). The $AURC/AUC_1$ has been shown to be a slightly less reliable marker in terms of individual stability than the AUC_G (AUC_G: $r = 0.520$, $p < 0.001$, $n = 31$ vs. AURC: $r = 0.336$, $p = 0.032$, $n = 31$; Edwards *et al.*, 2001a). The $AURC/AUC_1$ measures substantially depend on the first awakening sample, which may be subject to procedural issues consequent upon the difficulty of getting the timing for collection of this sample exactly right (e.g. difficulty for the participant appreciating exactly when they have actually woken up, not to mention discrepancies in the time between waking up and taking the first sample). Some authors (Wust *et al.*, 2000b) prefer to access a measure of the cortisol dynamic following awakening by calculating the mean increase[¶] (MnInc), this measure also relies heavily upon the first waking value and is also slightly less reliable than the AUC_G (AUC_G: $r = 0.630$, $p < 0.001$, $n = 509$ vs. MnInc: $r = 0.47$, $p = 0.001$, $n = 509$). However, there is close correspondence between the AUC_1/AURC and the MnInc (Edwards *et al.*, 2003), such that they are near identical in value and therefore interpretation. Others present a simple difference between waking and 30-min, or peak, values (Kunz-Ebrecht *et al.*, in press).

For clarity, examination of the ACR should include estimates of both total hormone secretory activity as well as the response. Shapes of the ACR can vary from high flat, low flat, low start with large increase and high start with large increase. At these early stages in the exploration of the significance of the ACR it is necessary to illustrate the raw data such that the shape of the curve as well as the derived formula can be examined. It may also be useful to distinguish ACR "responders" and "non-responders" based on the criterion that an increase of at least 2.5 nmol/l constitutes a response (Wust *et al.*, 2000b); however, it would be necessary to ensure that any non-response was not attributable to participant non-adherence. It is desirable that future published studies present correlates of the dynamic increase in cortisol secretion ($AURC/AUC_1$/MnInc or difference between maximum and minimum cortisol concentrations) as well as the overall cortisol secretory activity (AUC_G). These various ways of representing the ACR may be differentially associated with psychological well-being and health.

SIGNIFICANCE OF THE ACR

Regulatory Mechanisms

Examination of the relationship between the ACR and the rest of the cortisol diurnal cycle found that the dynamic of the awakening response (measured as AURC) was unrelated to the mean, underlying level of cortisol secretory activity throughout the rest of the day (Edwards *et al.*, 2001a). In addition, there is support for a genetic influence on the ACR but not on the remaining diurnal profile (Wust *et al.*, 2000a). Both of these pieces of evidence may imply that the ACR is under a regulatory influence independent of the rest of the diurnal cycle. The well-known associations between increasing age and cortisol secretion may also point in this direction: increasing age is linked with increased cortisol secretion throughout most of the day (e.g. Deuschle *et al.*, 1997a); however, the ACR is reported to be either not associated with, or negatively linked with, age such that increasing age is related to reduced cortisol secretion overall in the awakening period.

More evidence for the singular nature of the ACR is that it is sensitive to light. Under conditions of total darkness following awakening the ACR is still apparent but it can be significantly enhanced by 800 lux light exposure over the same post-awakening period (Scheer and Buijs, 1999). The same study demonstrated that evening cortisol levels were unaffected by exposure to light of the same luminescence. It thus appears that cortisol secretion is sensitive to light only in the morning. Indeed the effect of light on cortisol secretion in the morning can occur in the absence of the sleep-wake transition, as exposure to bright light in sleep-deprived participants (2000–4500 lux for 3 h) induced an immediate elevation of cortisol levels in the early morning but not in the afternoon (Leproult *et al.*, 2001). Recent studies from our laboratory have found that the ACR is sensitive to light manipulation even before awakening as the ACR can be enhanced by dawn simulation, a treatment for seasonal affective disorders which involves gradually increased light intensity for 30 min prior to awakening (Thorn *et al.*, in press). Thus, it seems that elevation of cortisol secretion in the morning depends upon both sleep–wake and dark–light cycles.

Taken together these studies imply a role in the regulation of the ACR for the hypothalamic supra-chiasmatic nucleus (SCN), which is light sensitive via afferents from the retina and plays an important role in synchronising the body's circadian rhythms. The direction of the association between awakening time and the ACR is in one sense counter-intuitive as it might be expected that later awakening, associated with brighter natural light, would be correlated with a greater ACR, but in fact the opposite has been reported (Edwards *et al.*, 2001b; Kudielka and Kirschbaum, 2003; Federenko *et al.*, in press), with no observations in the predicted direction. The observation that light does not influence cortisol secretion in the afternoon suggests a morning "window of opportunity" when the SCN is able to either drive the hypothalamic-pituitary-adrenal (HPA) axis centrally or increase adrenal sensitivity to ACTH. Perhaps later awakening is associated with reduced activity in one or both of these pathways.

[‡]AURC = sample 2 + s3 − (2*s1) + ((s4 − s2)/2).
[¶]MnInc = (sample 2 + s3 + s4)/3 − s1.

A direct neural pathway from the SCN to the adrenal cortex has been mapped in animal studies (Kalsbeek *et al.*, 1992; 1996; Buijs *et al.*, 1993; 1997; 1999; Dijkstra *et al.*, 1996) and appears to be the same in humans (Dai *et al.*, 1998). The sensitivity of the adrenal cortex to ACTH is greater in the ascending phase of the cortisol cycle (lights on in humans and lights off in rats) than during the rest of the day, an effect that can be abolished by ablation of the SCN (Buijs *et al.*, 1999; Sage *et al.*, 2001). This evidence points to a direct regulatory influence from the SCN upon the ACR, making it a discrete entity within the cortisol circadian cycle. The finding that there is a dissociation between ACTH and cortisol at the time of the morning peak (Fehm *et al.*, 1984; Spathschwalbe *et al.*, 1991; Born *et al.*, 1999) lends further support to this idea. Furthermore, normal diurnal fluctuations in plasma ACTH concentrations are low in amplitude so a direct influence of the SCN on the sensitivity of the adrenal cortex in the morning, or in the evening in nocturnal species, might be necessary to amplify the signal to achieve a characteristically pronounced cortisol rhythm (Sage *et al.*, 2001). Mechanisms by which adrenal innervation could influence adrenocortical steroidogenesis might be by modulating ACTH receptor sensitivity or by alteration in local blood flow thereby changing the delivery of ACTH to its receptors (see Sage *et al.*, 2001).

The Physiological Role of the ACR

The physiological role of the ACR has not been clearly defined. It was originally suggested that it might play an important metabolic role in mobilising energy reserves in the transition from sleep to wakeful physical activity (Pruessner *et al.*, 1997). More recently however, demonstration that the ACR is unrelated to blood glucose levels suggests otherwise (Hucklebridge *et al.*, 1999). In addition the ACR is independent of postural shift or normal ambulatory activity following awakening (Hucklebridge *et al.*, 2002). There is evidence to support the suggestion that a primary role of the ACR may be to switch the immune system from nighttime Th1 to daytime Th2 domination (Petrovsky and Harrison, 1997; Hucklebridge *et al.*, 1999). This hypothesis deserves direct investigation. Currently further insights into its physiological role can best be deduced by examination of its relationship with health (see below).

Relationships with Psychological Variables, Stress and Health

Research has demonstrated associations between the ACR and psychological well-being and physical health. However, it is unclear whether positive affect and health are consistently associated with larger or smaller awakening responses. For instance, a blunted ACR has been found to be associated with night time exposure to low frequency noise (Waye *et al.*, 2003), increasing age and a range of health problems (Kudielka and Kirschbaum, 2003), negative indexes of cardiovascular health in females (Eller *et al.*, 2001), and reduced bone density in premenopausal women (Brooke-Wavell *et al.*, 2002). However, in adults an elevated ACR has sometimes been associated with perceived stress (Schulz *et al.*, 1998; Steptoe *et al.*, 2000) but not always (Pruessner *et al.*, 1999; Kunz-Ebrecht *et al.*, in press). Similarly contradictory findings have been reported for burnout (Pruessner *et al.*, 1999; De Vente *et al.*, 2003). An increase in some aspects of the ACR (details in Table II) has been associated with non-clinical depression (Pruessner *et al.*, 2003b), lower grade of employment (Kunz-Ebrecht *et al.*, in press), loneliness (Steptoe *et al.*, in press), lack of social recognition (Wust *et al.*, 2000a), and self-reported upper respiratory symptoms (Edwards *et al.*, 2003). In addition, a heightened ACR has been observed in the juvenile offspring (age range 13–18 years) of parents with bipolar disorder (Ellenbogen *et al.*, in press).

Table II provides a brief summary of the reported associations between aspects of the ACR and psychosocial variables and health. It is difficult to make generalisations about the main findings. Many have found perceived stress and measures of negative affect to be linked to an increase in some aspect of the ACR, but this finding is not universal. In truth it is difficult to determine the significance of this mixture of findings when widely different methodologies have been employed and confounding factors have not been taken into account. It is not possible to undertake direct comparisons between studies or groups of participants as results are presented and analysed using a diverse range of strategies.

Summary

The ACR is a discrete and dynamic part of the circadian cortisol secretory cycle. Its main role and importance for health has yet to be fully elucidated, however early evidence suggests that it may be physiologically significant and be affected by stress. There are many discrepancies within the literature that may be attributable to a combination of procedural differences, confounding factors and non-adherence to protocol by participants.

To advance our understanding of this aspect of cortisol secretory activity it is desirable that future studies report on the major confounding factors (participant adherence, time of awakening, and type of day i.e. weekday–weekend, as well as age and gender). An additional possible confounding factor, associated with the sensitivity of the ACR to light, may be season, under circumstances when environmental light exposure markedly differs across seasons. Until this has been investigated and excluded it may also be valuable to include information about the time of year that the samples were collected. Although difficult the problem of participant adherence must be taken seriously and efforts made to exclude data where participant non-adherence is

TABLE II Associations between the ACR and psychosocial variables and health, showing the number of days over which the cortisol data were obtained and confounding variables not accounted for

Authors	Participants	Main result	Days studied, confounding factors not accounted for
Brooke-Wavell *et al.* (2002)	36 Healthy pre-menopausal f, 19–45 y, mean 30.9 y	Bone density positively correlated with cortisol concentrations at 0 and 30 min post-awakening	Single day, PA, L, WW
De Vente *et al.* (2003)	22 Burnout (14m, 8f, mean age 42) and 23 healthy (10m, 13f, mean age 31 y)	Burnout patients had higher overall cortisol levels in first hour post-awakening (mixed ANOVA: group × sampling time)	Single weekday, WT, PA, L
Edwards *et al.* (2003)	46 University personnel, 26f, 10m, 23–52 y, mean 34 y	High MnInc (but not AUC_G) associated with more URTI symptoms, but not perceived stress, in 2 weeks after sampling. High AUC_G associated with high perceived stress and more time spent in busy places	2 Days and inconsistent responders excluded ($n = 6$), WT, WW, L
Ellenbogen *et al.* (in press)	10 Offspring of bipolar parents (13–18 y, mean 15.6), 10 offspring of parents with no mental disorder (13–17 y, mean 14.7), 5f, 5m per group	Offspring of bipolar parents had higher cortisol in the first hour after awakening (mixed ANOVA: group × sampling time)	2 Days, PA, L, WW
Eller *et al.* (2001)	130 Public volunteers, 88f, 42m, 30–59 y, mean 44.5 y	In females higher cortisol 60 min post-awakening positively associated with a measure of cardiovascular health (reduced intima media thickness). No effect for males	Single weekday, WT, PA, L
Kudielka and Kirschbaum (2003)	179 Community dwellers 4–75 y, mean 29.6 y, of which 105 healthy	The 74 with health problems had attenuated AURC compared to healthy	Single day, WT, L, PA, WW
Kunz-Ebrecht *et al.* (in press)	227 Government employees, 45–59 y, mean 52.2 y	Greater increase in cortisol (from 0–30 min post-awakening) for lower employment grade. No association with subjective ratings of stress (weekend vs. weekday differences discussed in confounding factors section above)	Single weekday and 1 weekend day, excluded if self-reported WT > 10 min before first sample (7.3%), L
Pruessner *et al.* (1999)	66 Teachers, 42f, 24m, mean age 43.6 y	High burnout ($n = 30$) had lower cortisol levels after awakening (mixed ANOVA). Perceived stress not associated with ACR except after DEX suppression when high stress associated with bigger increase in cortisol (interaction of stress × time) in contrast burnout linked with lower cortisol after DEX (burnout × time).	2 Weekdays + 1 weekday with DEX, PA, L, WT
Pruessner *et al.* (2003b)	40 m Students, 18–35 y, mean 24.3 y	Correlation between median aggregated AUC_G and sub-clinical depression ($n = 33$). Median split for depression (all non-clinical severity) showed sig group by time interaction with cortisol at 30 and 60 min post-awakening (not 0 min) being higher with high depression scores	One weekday/week for 4 weeks PA, WT, L
Schulz *et al.* (1998)	Students, $n = 100$, 51f, 49m, 19–55 y, mean 25.7 y	48 low (L) vs. 37 high (H) chronic work overload group difference (mixed ANOVA): H had higher levels at 30, 45 and 60 min. Greatest effect in f	3 Days, PA, WT, L, WW
Steptoe *et al.* (in press)	240 Government employees, 111f, 129m, 45–58 y, mean 52.3 y	High loneliness positively related to the cortisol increase from 0–30 min post-awakening, and the AUC_G	Single typical working day, excluded if self-reported WT > 10 min before first sample, L
Waye *et al.* (2003)	12 m Students, mean 24.5 y	Night time exposure to low frequency noise caused an attenuated ACR (within subjects ANOVA), reduced cortisol at 30 min post-awakening	5 Consecutive weekdays, PA, L, WT
Wust *et al.* (2000a)	52 MZ, 52 DZ twin pairs, 8–64 y, mean 19.6 y	Increased with high worries, social stress and lack of social recognition (all sig, time by variable interactions after median split for group). No link with self-esteem, or self-efficacy (hereditability findings discussed in regulatory mechanisms section above)	2 Weekend days, PA, WT, L

The studies are listed in alphabetical order by first author. f, female; m, male; y, years; MZ, monozygotic; DZ, dizygotic; sig, statistically significant; AUC_G, area under the ACR curve relative to ground or zero; AURC, area under the ACR curve relative to the first waking sample; MnInc, mean increase in cortisol relative to the first waking sample; DEX, Dexamethasone; PA, participant adherence; WT, awakening time; L, light; WW, weekday/weekend.

suspected. It is not adequate to simply ask people whether they have accurately complied with the procedural protocol. It is suggested that sampling on two consecutive similar days allows examination of consistency of individual responses over the 2 days. All available data suggest that individual responses across two such days should be similar and major deviations from this would be indicative of non-compliance. Alternatively an electronic monitoring device could be employed, although this still cannot control for the accurate collection of the first awakening sample.

The ACR is a complex phenomenon and deviations from the "norm" can take various forms, e.g. changes in the increase of cortisol secretion, the total level of cortisol secretory activity and/or the duration of the ACR. In order to understand published data adequately the reader requires access to the shape of the response as well as both derived composites i.e. overall level of secretory activity (e.g. AUC_G) and the dynamic of the response (e.g. AURC/AUC_1 or MnInc).

It is early days in the search for the role and significance of the ACR. Despite some inconsistencies in the literature it is clear that this discrete aspect of the cortisol circadian cycle is a major aspect of neuroendocrine activity. Its putative role in regulation of physiological function across the day (e.g. modulation of immune system activity) and its sensitivity to psychosocial variables makes it a prime candidate as an intermediary linking mind and health.

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