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The effect of dawn simulation on the cortisol response to awakening in healthy participants

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Summary Bright light exposure after awakening has been shown to elevate cortisol levels in healthy participants. The present study examined the effect of dawn simulation (a treatment for seasonal affective disorder) on the cortisol response to awakening and mood. Twelve healthy participants were supplied with a dawn simulator (The Natural Alarm Clock, Outside In, Cambridge Ltd), a bedside light that increases in intensity prior to awakening to approximately 250 lux over 30 mins when an audible alarm sounds. A counterbalanced study was performed on 4 consecutive normal weekdays, two of which were control days (no dawn simulation) and two experimental (dawn simulation). Saliva samples were taken immediately on awakening then at 15, 30 and 45 minutes post awakening on all 4 study-days. Total cortisol production during the first 45 mins after awakening was found to be significantly higher in the experimental condition than in the control condition. Participants also reported greater arousal in the experimental condition and there was a trend for an association between increased arousal and increased cortisol secretory activity under dawn simulation. This study provides supportive evidence for the role of light and the suprachiasmatic nucleus in the awakening cortisol response.

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1. Introduction

In healthy individuals cortisol secretory activity shows a marked diurnal rhythmicity characterised by peak levels following awakening and a declining pattern thereafter (Edwards et al., 2001a). The cortisol response to awakening is well documented (Pruessner et al., 1997; Wuest et al., 2000b; Edwards et al., 2001a). Typically, there is

a 50–150% increase in free cortisol, as measured in saliva, peaking at 30–45 mins post-awakening. This phase of the diurnal cortisol cycle has a moderate to high within-subject stability (Wuest et al., 2000b; Edwards et al., 2001a) and is unrelated to cortisol secretory activity over the remainder of the diurnal cycle (Edwards et al., 2001a). In addition there is evidence for a genetic influence on the awakening cortisol response but not on the remaining diurnal profile (Wuest et al., 2000a) implying that it is under an independent regulatory influence. Indeed the hypothalamic pituitary adrenal (HPA) axis cannot be the sole

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pathway involved as there is a dissociation between ACTH and cortisol at the time of the morning peak (Fehm et al., 1984; Späth-Schwalbe et al., 1991; Born et al., 1999).

The awakening cortisol response can be modulated by light exposure following awakening. Under conditions of total darkness over the first hour following awakening the awakening cortisol response is still apparent but can be significantly enhanced by 800 lux light exposure over the same post awakening period (Scheer and Buijs, 1999). In the same study evening cortisol levels were unaffected by light. The effect of light on cortisol in the morning can occur in the absence of the sleep-wake transition (Leprout et al., 2001). Exposure to bright light in sleep-deprived participants (2000–4500 lux for 3 hrs) induced an immediate elevation of cortisol levels in the early morning but not in the afternoon. Thus, cortisol elevation in the morning depends upon both sleep-wake and dark-light cycles. The phase-dependent effect of light on cortisol is thought to be mediated by the suprachiasmatic nucleus (SCN) since the influence of light on plasma corticosterone levels in the rat depends upon the integrity of the SCN as well as circadian phase (Buijs et al., 1999).

Further evidence for the influence of light on neuroendocrine function can be found in research on seasonal affective disorder (SAD) and light therapy. SAD is characterised by affective illness in autumn/winter and non-depressed periods in spring/summer (Rosenthal et al., 1984). Bright light therapy has been shown to be effective in the treatment of SAD (Terman et al., 1989b). There is evidence that circadian rhythms of melatonin, core body temperature and cortisol are phase-delayed in SAD and normalised by bright light therapy (Lewy et al., 1987; Avery et al., 1997). Dawn simulation, low intensity light that gradually increases in luminance before the participant awakes (Terman et al., 1989a), has been shown to be at least as effective as bright light post awakening in alleviating symptoms of depression in SAD (Avery et al., 2001). However, no published study to date has examined the effect of dawn simulation on the cortisol response to awakening in either healthy participants or those with SAD. The aim of the present study was to investigate the effect of dawn simulation on the cortisol response to awakening and mood in healthy participants. It was hypothesized that dawn simulation will enhance the awakening cortisol response, as has been demonstrated for light exposure during the immediate post-awakening period.

2. Methods

2.1. Participants

Twelve participants were recruited from among the academic community at the University of Westminster and informed written consent given. Participants were five females and seven males, mean age 39, ranging from 24–54 years. Participants were recruited on the basis that they were not taking medication, had no chronic or acute illness at the time of recruitment and that their habitual weekday waking time was 0700 h or earlier (i.e. at least half an hour before dawn during the period of investigation). Participants received no direct financial incentive but entry into a small prize draw was offered as a reward for completing the study.

2.2. Materials

Participants were supplied with a 'Natural Alarm Clock' (Outside In, Cambridge Ltd), a low intensity bedside light that gradually increases in luminance to approximately 250 lux over 30 mins before awakening when an audible alarm sounds. Participants were also provided with a study pack containing full standardised written instructions and a saliva sampling kit, consisting of four re-sealable plastic bags labelled DAY 1–4 containing a record sheet, stress arousal questionnaire, 4 short plastic disposable straws and 4 numbered Eppendorf tubes (1–4).

2.3. Procedure

The study took place during the months of January and February in the UK. The study was performed on 4 consecutive, normal weekdays, two days being control and two experimental (counterbalanced). As well as standardised written instructions, participants received verbal explanations of the procedure and a demonstration of the use of the Natural Alarm Clock. On the night before commencement of the study participants were instructed to place the Natural Alarm Clock beside their bed in place of their usual bedside light.

On control days participants were asked to set their usual alarm clock to wake them and on experimental days participants set the Natural Alarm Clock to wake them. They were asked to record if they awoke before the alarm sounded during dawn simulation. If they woke before dawn simulation started in the morning they were asked not to take saliva samples and to repeat the procedure on the next convenient weekday.

Participants were instructed to take saliva samples immediately on awakening then at 15, 30 and 45 mins post awakening on all 4 study days by dribbling through a straw into an Eppendorf tube. During the saliva collection period participants were instructed to take nil by mouth bar water, and not to smoke or brush their teeth to avoid abrasion and vascular leakage. Samples were stored in the participants' home freezer as soon as possible after collection of saliva and transferred to the laboratory in insulated cold packs and stored at -20°C until assay. Participants were asked to fill in a record sheet on each day, recording awakening time and time of collection of saliva samples. In addition, participants filled in a stress-arousal checklist (Mackay et al., 1978) on each day after collection of the 45 min saliva sample. Other than these instructions participants were asked to follow their normal routine. These general procedures for measuring the awakening cortisol response within the domestic setting have been adopted in numerous published studies.

2.4. Cortisol assay

Samples were thawed and centrifuged at 3500 rpm for 10 mins. Cortisol concentration was determined by Enzyme Linked Immuno-Sorbent Assay developed by Salimetrics LLC (USA). Sensitivity: 0.19 nmol/l (lower limit). Standard range in assay: 0.19–49.0 nmol/l. Correlation of assay with serum: $r = 0.960$, $p < 0.0001$, $n = 19$ samples. Intra and inter-assay variations: 3.9 and 6.7% respectively.

3. Results

Time of awakening was very similar across all four study days. Mean time of awakening on control days was 0640 h and on experimental days was 0633 h. Control day awakening time was significantly positively correlated with experimental awakening time ($r = 0.947$, $p < 0.0005$, two-tailed). All participants awoke before 0700 h and the earliest sunrise during the study period was 0730 h so participants awoke in the absence of daylight. No participant had acute illness during the study. No participant awoke prior to the dawn simulation, therefore the contingency of repeating procedures did not arise. Six out of the 12 participants awoke prior to the alarm sounding during dawn simulation on at least one of the experimental days. Mean exposure to dawn simulation therefore ranged between 20 mins and the maximum of 30 mins. Participants reported that they collected saliva samples at the correct times.

Cortisol concentrations in this study ranged between 1 and 40 nmol/l and were moderately

skewed. A square root transformation was performed, which normalised sample distributions and reduced skewness statistics.

Four-way mixed ANOVA (condition [2] \times day [2] \times sampling time [4] \times order (between subjects) [2]) was performed on the cortisol data. A significant main effect of sampling time demonstrated the expected awakening response ($F_{(2,491, 24.907)} = 14.193$, $p < 0.0005$; Greenhouse-Geisser corrected), on all four study days there was a marked increase in cortisol concentration following awakening, peaking on average at 45 mins. Thus, the general awakening pattern of cortisol for the sampling period was in line with what has been reported widely in the literature.

The same analysis revealed a significant main effect of dawn simulation ($F_{(1,10)} = 5.815$, $p < 0.05$). Total cortisol production for the entire period following awakening was significantly higher in the experimental condition than in the control condition. Gradually increasing low intensity light prior to awakening increased participants' awakening cortisol responses (see Fig. 1(a)). There were no order effects and no significant interactions between any of the factors.

Overall cortisol production during the first 45 mins following awakening was also calculated for each day as the area under cortisol curve with reference to zero (AUC) in order to evaluate the effect further. AUC did not differ across the two control days or across the two experimental days. Furthermore, AUC was stable across the two control days ($r = 0.637$, $p < 0.05$, two-tailed) and across the two experimental days ($r = 0.836$, $p < 0.005$, two-tailed). Mean control AUC and mean experimental AUC were computed and, confirming the ANOVA result, a paired t -test revealed that cortisol secretory activity as measured by AUC was significantly greater under dawn simulation ($t = 2.492$, $df = 11$, $p < 0.05$, two-tailed). Fig. 1(b) illustrates a case-by-case comparison of the mean control day and mean experimental day AUCs.

Overall cortisol production was greater in the experimental condition than control as indicated by the above analyses. Fig. 1(a) indicates that the difference is most apparent at the 15 and 30 min samples. However, we had no specific hypotheses regarding timing and there was no significant interaction (sample \times condition) so any further analysis needs to be interpreted cautiously. Nevertheless, after Bonferroni correction the difference at 15 mins was close to significance but not at any other sampling point. The peak value was similar in both conditions at 45 mins.

The average % increase in AUC from the control to the experimental condition was 12.8%. The

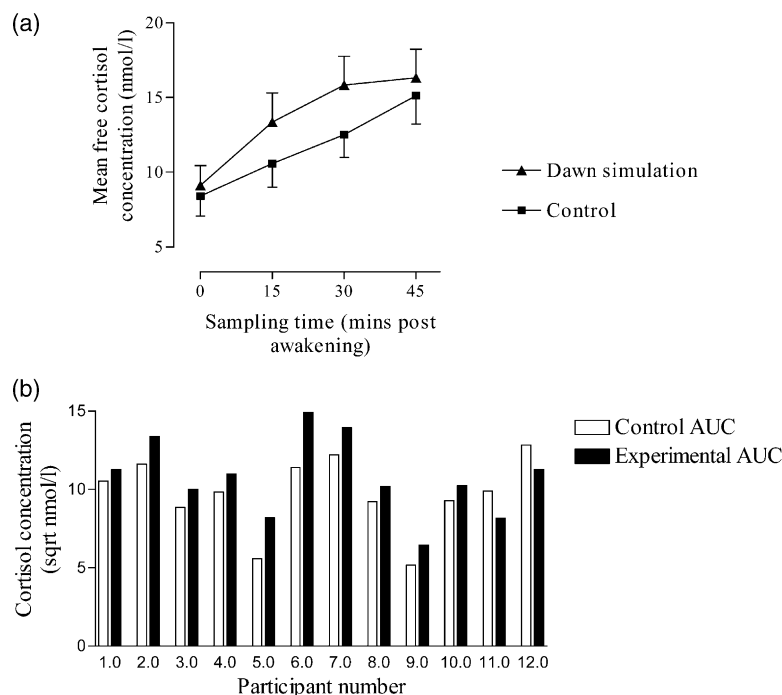


Fig. 1. (a) Mean (\pm SEM) free cortisol concentrations (nmol/l) for control and experimental (dawn simulation) conditions ($N = 12$). (b) Area under cortisol curve with reference to zero (AUC; SQRT nmol/l) for control and experimental (dawn simulation) conditions for each participant ($N = 12$).

effect was also highly consistent, with only two of the 12 participants showing decreased AUCs in the experimental condition compared to the control. As 6 participants awoke during the dawn simulation period on at least one of the experimental days, mean times of exposure to dawn simulation in minutes were correlated with AUC difference scores between control and experimental conditions. However, no significant relationship was found ($r = -0.205$, $p = 0.523$).

The stress-arousal questionnaires were scored in accordance with guidelines (Mackay et al., 1978). Wilcoxon Matched Pairs Signed Ranks tests revealed that participants reported increased arousal on experimental days compared with control days ($z = 2.608$, $p < 0.001$, 2-tailed). There was no difference in self-reported stress ($z = 1.840$, $p = 0.066$, 2-tailed) (Fig. 2.)

Further analyses were undertaken to examine whether increased arousal was associated with increased cortisol production under dawn simulation. Although the statistical power is extremely limited for this kind of post-hoc analysis, Spearman's correlation revealed a non-significant trend in the predicted direction ($\rho = 0.457$, $p = 0.135$, two-tailed).

4. Discussion

The results of this study demonstrate primarily an enhancement of the awakening cortisol response after exposure to dawn simulation prior to awakening. The expected increase in cortisol secretion during the first 45 mins post awakening occurred on all 4-study days, even in the control condition when participants awoke prior to natural dawn. That the awakening response occurs in darkness has been demonstrated previously (Späth-Schwalbe et al., 1991; Scheer and Buijs,

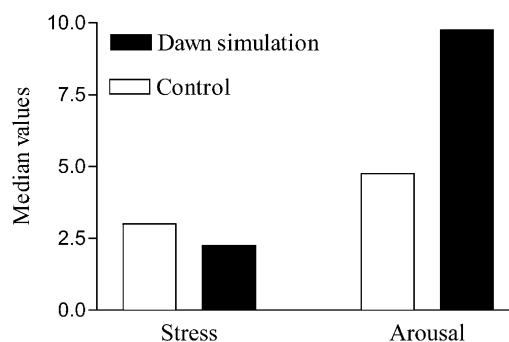


Fig. 2. Median stress and arousal scores derived from the Stress-Arousal Checklist (Mackay et al., 1978) for control and experimental (dawn simulation) conditions ($N = 12$).

1999) indicating that the sleep-wake transition alone is a major input to the awakening response.

However total cortisol production for 45 mins following awakening was significantly higher in the dawn simulation condition than in the control condition. This effect appeared to be due to an enhanced rate of cortisol increase following awakening without affecting peak levels—within the sampling period. In future studies it would be interesting to explore whether dawn simulation accelerates the awakening cortisol response rather than affecting overall levels. This could be achieved by expanding the sampling period to determine whether awakening in the dark is associated with a later peak. As no comparison was made between stable light exposure and dawn simulation we cannot conclude that it was the gradually increasing nature of the light that caused the effect.

The findings are consistent with the evidence that light exposure during the post awakening period increases early morning cortisol levels (Scheer and Buijs, 1999) and that early morning exposure to bright light elevates cortisol in the absence of sleep-wake transition (i.e. when participants had been sleep deprived) (Leproult et al., 2001). These two studies utilised light intensities of 800 lux over 1 hr and 2000–4500 lux over 3 hrs respectively. The light intensity used in the current study was at its maximum only 250 lux and incident upon the retina via the shut eye. However, these naturalistic dawn exposures prior to awakening have been found to alleviate the symptoms of depression in SAD (Avery et al., 2001). Furthermore, other authors have found that relatively low levels of light can influence the human circadian timing system as indicated by core body temperature (Boivin et al., 1996; Boivin and Czeisler, 1998).

The SCN, which synchronises neuroendocrine secretory activity in relation to the light-dark cycle, is likely to be implicated in the effect of light on the cortisol response to awakening. Several studies have provided evidence for a direct multi-synaptic neural pathway from the SCN to the adrenal cortex (Kalsbeek et al., 1992; Buijs et al., 1993; Dijkstra et al., 1996; Kalsbeek et al., 1996; Buijs et al., 1997; Buijs et al., 1998; Buijs et al., 1999). That this pathway forms a regulatory mechanism contributing to the awakening cortisol response in addition to a SCN-HPA pathway is suggested by the evidence that there is a dissociation between ACTH and cortisol at the time of the morning peak (Fehm et al., 1984; Späth-Schwalbe et al., 1991; Born et al., 1999). The mechanisms by which adrenal innervation could influence ster-

oidogenesis might be by regulation of adrenocortical sensitivity to ACTH or by direct ACTH-independent neural regulation (for a review see Ehrhart-Bornstein et al., 1998).

A phase-dependent effect of light upon cortisol secretory activity has been demonstrated as levels in the afternoon or evening are not affected by light exposure (Scheer and Buijs, 1999; Leproult et al., 2001). Furthermore, bright light therapy for the treatment of SAD is more effective when administered in the morning rather than later in the day (Lewy et al., 1998; Terman et al., 2001). These studies provide evidence that the early morning is when the SCN-adrenal cortex pathway is most responsive to light. This may explain the finding that the awakening cortisol response is unrelated to cortisol secretory activity over the remainder of the diurnal cycle (Edwards et al., 2001a). Although data in the present study could not contribute to the evidence that the cortisol response to awakening is influenced by time of awakening (as there was very little variance in between-subject wake time) other studies have shown that early awakening is associated with an enhanced cortisol response (Edwards et al., 2001b; Kudielka and Kirschbaum, 2003). It is conceivable that early awakening initiates a stimulatory input to the SCN-adrenal pathway when it is at its most sensitive.

A further finding of the present study was that participants reported greater arousal measured by the stress-arousal checklist (Mackay et al., 1978) in the dawn simulation condition. Participants reported that they felt more alert and less tired when using the dawn simulator to wake them in the morning rather than their usual alarm clock. Self-reported stress levels, however, were not different in control and experimental conditions. Although these self-report measures might be influenced by demand characteristics (participants were aware of experimental condition) the finding is in line with Leproult et al. (2001) who found that light limited the deterioration of alertness usually associated with sleep deprivation. Although the awakening cortisol response has been demonstrated to be unrelated to blood glucose levels at time of awakening, and is therefore not thought to be primarily related to the mobilisation of energy reserves (Hucklebridge et al., 1999), it may play a role in cognitive arousal. The trend for an association between increased arousal and increased cortisol secretory activity under dawn simulation suggests a psychophysiological association and supports the self-report findings.

This study demonstrates for the first time that gradually increasing low-level illumination prior to

awakening increases the cortisol response to awakening. The influence of light and the implied involvement of the SCN suggest that the awakening cortisol response is a discrete aspect of the cortisol circadian cycle.

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