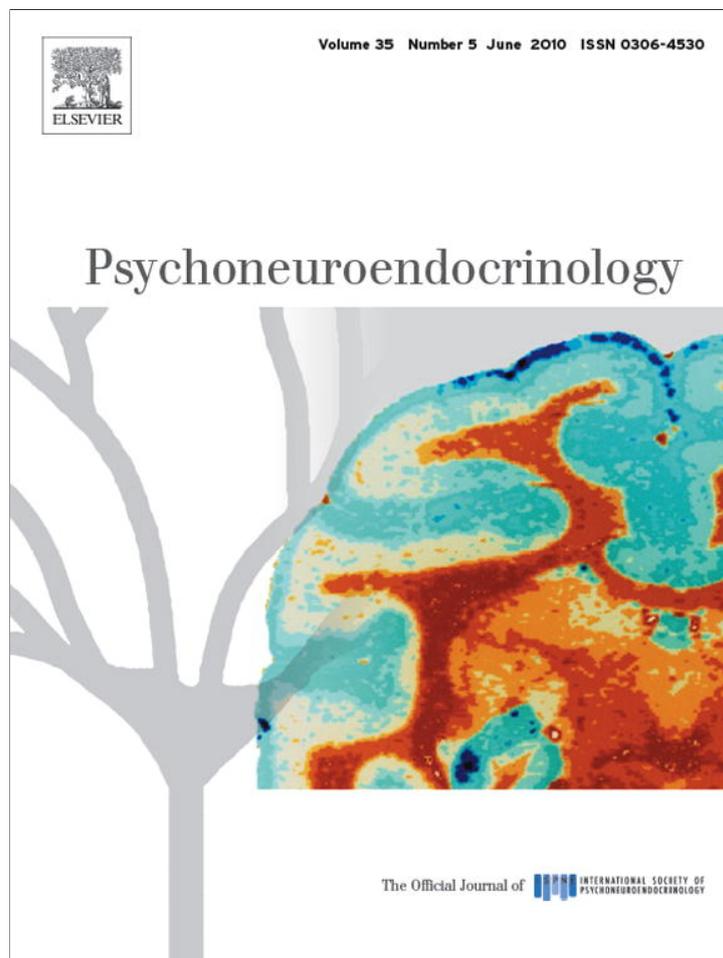


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Cortisol awakening response (CAR)'s flexibility leads to larger and more consistent associations with psychological factors than CAR magnitude

Moira Mikolajczak^{a,1,*}, Jordi Quoidbach^{b,1}, Valentine Vanootighem^{b,1},
 Florence Lambert^{b,1}, Magali Lahaye^{c,1}, Catherine Fillée^{d,1},
 Philippe de Timary^{e,1}

^a Belgian National Fund for Scientific Research (FNRS), Université catholique de Louvain, Department of Psychology, Place Cardinal Mercier 10, B-1348 Louvain-La-Neuve, Belgium

^b University of Liège, Department of Psychology, Boulevard du Rectorat 5 (Bldg 32), B-4000 Liège, Belgium

^c Université catholique de Louvain, Department of Psychology, Place Cardinal Mercier 10, B-1348 Louvain-La-Neuve, Belgium

^d Université catholique de Louvain, Cliniques Universitaires Saint Luc, Department of Medical Biochemistry, Avenue Hippocrate 10, 1200 Bruxelles, Belgium

^e Université catholique de Louvain, Cliniques Universitaires Saint Luc, Department of Neurology and Psychiatry, Avenue Hippocrate 10, 1200 Bruxelles, Belgium

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 Stress

Summary The cortisol awakening response (CAR) is increasingly recognized as a potential biological marker of psychological and physical health status. Yet, the CAR literature is replete with contradictory results: both supposedly protective and vulnerability psychosocial factors have been associated with both increased and decreased CAR. In this study, we tested the hypothesis that the CAR *flexibility* would be a better indicator of psychological status than CAR *magnitude*. Forty-two men measures of happiness, perceived stress and neuroticism, and took saliva samples immediately on awakening, then at 15, 30, 45 and 60 min post-awakening on three study days (i.e., Sunday, Monday and Tuesday). When considering the CAR magnitude, our effects perfectly reflect the inconsistencies previously observed in the literature (i.e., the main effects of the psychological predictors are not consistent with each other, and the effect of one predictor on a given day contradicts the effect of the same predictor on another day). However, considering the CAR flexibility leads to a fully consistent pattern: protective factors (i.e., high happiness, low stress, low neuroticism) are associated with a flexible CAR (i.e., lower CAR during weekends compared to workdays) whereas

* Corresponding author. Tel.: +32 10 47 44 09.

E-mail address: Moira.Mikolajczak@uclouvain.be (M. Mikolajczak).

¹ All authors have contributed equally to this paper.

vulnerability factors (i.e., low happiness, high stress, high neuroticism) are associated with a stiff CAR (i.e., same magnitude during weekends and workdays). We conclude that considering the CAR *flexibility* (e.g., between weekends and workdays) rather than the traditional CAR *magnitude* might be a way to understand the apparent conflicts in the CAR literature.

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1. Introduction

In order to survive, human beings need to be able to flexibly adjust to changing environmental demands. The hypothalamo-pituitary-adrenal axis (HPA axis) is a crucial system to that end: triggered by challenges, it induces a number of bio-behavioral changes that help the organism preserving homeostasis (e.g., Tsigos and Chrousos, 2002). It has been suggested that the adrenocortical activity could be indexed by the free cortisol response to awakening (see e.g., Pruessner et al., 1997; Wust et al., 2000; Clow et al., 2004; Fries et al., 2009). The cortisol awakening response (CAR) refers to the sharp increase in cortisol release observed over the first 30 min after awakening. Whereas the exact role of the CAR has still not been fully clarified, Adam et al. (2006) have suggested that it is an adaptive response aimed to provide the individual with the boost needed to meet the anticipated demands of the upcoming day. In the same vein, Fries et al. (2009) have suggested that the CAR may accompany an activation of prospective memory representations at awakening, enabling individuals to orient themselves in space and time, and helping them anticipate upcoming demands. Preliminary evidence tends to corroborate this “anticipation” hypothesis. For instance, a single case study by Stalder et al. (2009) shows a positive relationship between the CAR and study-day anticipations of the level of obligations. It has also been observed that the CAR is lower on weekends compared with workdays (e.g., Schlotz et al., 2004) and lower on “normal days” compared with days where a tournament is planned (Rohleder et al., 2007). Building on these findings, we reasoned that the CAR’s variations according to situational demands (i.e., the CAR flexibility) should be a particularly good indicator of the HPA axis quality of functioning.

So far, the literature has mainly focused on the CAR magnitude (see e.g., Schlotz et al., 2004 for exception). The typical procedure involves aggregating multiple CAR measures across two or more weekdays into an overall CAR magnitude index. These days did not a priori differ regarding expected demands or were at least treated as such (i.e., responses across days were aggregated, rather than compared). The rationale behind the focus on the CAR magnitude is that waking-up is a challenge to which a healthy HPA axis has to respond. However, little is known about how a healthy axis should respond. A hundred of published studies on the CAR have left this question unanswered. Both increased and decreased CARs have been associated to both good and bad somatic and psychological conditions. As elegantly summarized by Fries et al. (2009), patients displayed *higher* CAR than controls in some studies (e.g., Edwards et al., 2003; Lieb et al., 2004; Steptoe et al., 2004; Therrien et al., 2007) and *lower* CAR than controls in other studies (e.g., Kudielka and Kirschbaum, 2003; Bohmelt et al., 2005; De Kloet et al., 2006; Wirtz et al., 2007). Even more puzzling, the same psychological conditions have been associated with an increased CAR in some studies and a decreased CAR in others

(see the recent meta-analysis by Chida and Steptoe, 2009). Far from being an isolated phenomenon, contradictory results have been observed on key variables, ranging from psychological state (e.g., stress, negative affect) to psychological disorders (e.g., burnout, depression), and personality traits (e.g., neuroticism, trait anxiety). These contradictions result in very small aggregated effects sizes, usually comprised between $-.01$ and $.01$ (Chida and Steptoe, 2009).

Thus, although many studies demonstrate a significant association between CAR and various psychological or somatic conditions, their results are currently hardly usable because it is still unclear what a “healthy” CAR should look like. This is highly problematic because it makes it impossible to *predict* a person’s psychological and/or health status on the basis of his/her CAR. Likewise, it is also impossible to *explain* disorders by the different shapes of their associated CAR as (1) this shape is inconsistent across studies and (2) it is unknown what a healthy shape should look like.

What we know for sure, however, is that the HPA axis—and cortisol in particular—is meant to help the organism to deal with challenges. Thus a healthy HPA axis should be able to flexibly adapt to higher and lower environmental demands. Fries et al. (2009) have concluded from their review of available evidence that the cortisol rise after awakening reflects the anticipation of the next day’s demands. Accordingly, normal subjects show a modulation of the CAR, such that the higher the demands, the higher the CAR (e.g., Schlotz et al., 2004). It is thus expected that the dysregulation of the HPA axis associated with poor psychological indicators would manifest in a loss of flexibility. That is, the CAR would fail to vary according to circumstances, thereby failing to adjust the organism energetic supply to situational demands. In the current study, we tested the hypothesis that the CAR *flexibility* (i.e., difference between weekends and workdays CARs: $\Delta\text{CAR}_{\text{we-wd}}$) would be a better indicator of psychological state than CAR *magnitude* (i.e., CAR with respect to the ground or the increase). Therefore, we selected three psychological variables for which conflicting results were observed: one “positive variable” (happiness) and two “negative variables” (perceived stress and neuroticism). We examined then how these variables related to CAR magnitude and CAR flexibility. If our hypothesis is correct, inconsistent, weak or null relationships should be observed between our three predictors and the CAR magnitude, while a coherent and strong pattern of finding should emerge using the CAR flexibility.

2. Methods

2.1. Participants

Participants were recruited among full-time employees of companies located nearby the campus of University of Liège. Participants were eligible for participation if they were men (in order to avoid gender differences in CAR; Wust et al.,

2000), between 30 and 50 years old, healthy (i.e., no medication, no somatic or psychiatric disorder), non-smokers, and willing to give 2 h to scientific research. Fifty interested participants attended briefing sessions, comprising detailed verbal information about the requirements of the study. As in Thorn et al. (2009), participants were informed that failure to respect the guidelines would affect the results of the study. In order to improve adherence, participants were told that previous research had shown that non-adherence was reflected in the cortisol profile, and that they would not be paid if their profile failed to demonstrate adherence. Participants were also shown how to collect saliva. Eight participants withdrew after being presented with the study requirements. A total of 42 participants ($M_{\text{age}} = 38$, $SD = 6.29$) completed the study and received 30€ in exchange for their participation. The demographics and health questionnaire confirmed that all participants were free from somatic or psychiatric illness and that none was on drug or medication susceptible to affect cortisol measures.

2.2. Procedure

The protocol was approved by the ethics committee of the Psychology Department of the University of Louvain. The experimenter met each participant at their workplace and gave them the salivettes[®] sampling device (Sarstedt, Germany), the set of questionnaires (see Section 2), and a brief reminder of the saliva sampling protocol. Awakening cortisol levels were assessed at home on three consecutive days (Sunday, Monday and Tuesday) using the Salivettes[®]. Subjects were instructed to collect samples 0, 15, 30, 45 and 60 min after awakening. Samples were stored in participants' freezers before being transferred to the laboratory where they were stored at -20°C until assay. In addition, participants were required to answer a quick questionnaire each morning, recording time of awakening and sleep quality (1 very poor to 5 very good).

3. Measures

Happiness was measured through the Subjective Happiness Scale (Lyubomirsky and Lepper, 1999). This well-validated instrument composed of four 7-point items provides a global, subjective assessment of whether one is a happy or an unhappy person ($\alpha = .86$).

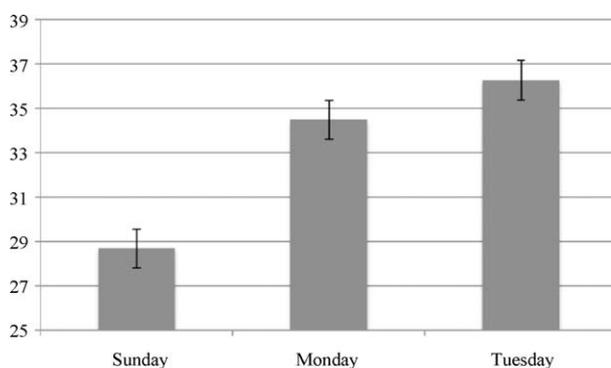


Fig. 1 Effect of measurement day on mean cortisol awakening response (expressed in nmol/l).

Perceived stress was evaluated using the Perceived Stress Scale (PSS-10; Cohen et al., 1983; Cole, 1999). This questionnaire comprises 10 items rated on a 5-point scale ($\alpha = .89$).

Neuroticism was appraised through the neuroticism subscale of the "International Personality Item Pool" scales (IPIP-50, Goldberg et al., 2006), which is a widely used personality inventory based on the Five Factor Model (FFM; Costa and McCrae, 1992). This questionnaire assesses the big five dimensions of neuroticism, extraversion, openness, conscientiousness, and agreeableness through 50 items rated along a 5-point scale (1 = does not describe me at all to 5 = describes me perfectly). The neuroticism subscale was composed of 10 items ($\alpha = .83$).

4. Statistical analyses

All analyses were performed using SPSS 15. One person failed to return the questionnaires, leaving 41 subjects for the analyses.

To examine the overall association of age, Body Mass Index (BMI), marital status, number of children, sleep quality and wake-up time with the CAR, two cortisol parameters were computed for each participant on each collection day. The area under the curve with respect to ground (CAR_g) and the area under the curve with respect to the increase (CAR_i) (see Pruessner et al., 2003 for the formulas). These indexes were then correlated with the aforementioned variables.

The correlations among the various psychological predictors (happiness, perceived stress and neuroticism) were examined through Pearson Correlations.

To test for the influence of measurement days and sampling time on cortisol, we performed a mixed-model on cortisol concentrations, with day and time being a within-subject factor, and subject being a random factor. Because wake-up times were found to have an independent influence on the CAR, we included waking hours as covariates in the model.

To determine the influence of measurement day and psychological predictors (happiness, perceived stress and neuroticism) on cortisol responses, we performed three mixed-models (1 for each psychological predictor) on cortisol concentrations, with day being a within-subject factor, psychological predictor being a between-subject factor, and subject being a random factor. Because wake-up times were found to have an independent influence on the CAR, we included waking hours as covariates in the models.

In order to illustrate significant effects of continuous variables, psychological predictors were then dichotomized (above/below the mean) and plotted. Thus, the graphs in Fig. 1 represent effects obtained on categorical variables disregarding the influence of covariates, whereas the results of the statistical analyses reported in the text and in Table 1 represent the effects of the continuous variables and are covariate adjusted.

5. Results

5.1. Influence of BMI, marital status, number of children, sleep quality, and wake-up time on CAR

We found the CAR to be unrelated to age, BMI, marital status, number of children, and reported sleep quality. However, the CAR was significantly related to wake-up time. Correlations

Table 1 Main and interaction effects (F - and p -value) of measurement day and psychological factors on CAR.

Type of effect	Happiness	Neuroticism	Perceived stress
Main effect of day	7.30 ^{***}	16.95 ^{***}	22.80 ^{***}
Main effect of predictor	3.20 ^{***} (happiness = ↓CAR)	7.46 ^{***} (neuroticism = ↑CAR)	8.61 ^{***} (stress = ↓CAR)
Interaction effect day × predictor	2.59 ^{***} (see Fig. 2)	1.73 ^{**} (see Fig. 2)	2.03 ^{***} (see Fig. 2)

Note. The first row shows that the magnitude of the CAR depends on the day of measurement (main effect significant in all models). The second row shows that the CAR varies according to the level of happiness, stress and neuroticism of the subject (direction of the effect included in brackets). The third row indicates that the difference of magnitude between weekdays and weekends (i.e., the CAR flexibility) depends on psychological factors (see Fig. 2). Note that wake-up times were entered as covariates in the model.

^{**} $p \leq .01$.
^{***} $p \leq .001$.

between wake-up time and CAR_g were $-.43$ on Sunday ($p < .05$), $-.35$ on Monday ($p < .05$) and $-.15$ on Tuesday (ns). Correlations between wake-up time and CAR_i were $-.39$ on Sunday ($p < .05$), $-.03$ on Monday (ns) and $-.34$ on Tuesday ($p < .05$).

5.2. Influence of measurement day and sampling time on CAR

Mixed-models yielded first a significant effect of sampling time ($F = 5.46$, $p \leq .001$): participants showed a sharp increase in cortisol concentrations over the first 30 min after awakening, followed by a progressive decrease. There was also a significant effect of day ($F = 23.44$, $p \leq .001$), with average cortisol concentrations being higher on weekdays than on weekends (see Fig. 1). There was no significant interaction effect ($F = 1.74$, ns). Thus, our participants showed the expected profiles (i.e., there was a curve on each day), and behaved according to previous studies (i.e., the magnitude of the CAR was higher during the week).

5.3. Correlations among psychological factors

As expected, the correlations among neuroticism, stress and happiness were high: happiness was negatively correlated with both perceived stress ($r = -.58$, $p \leq .001$) and neuroticism ($r = -.66$, $p \leq .001$), which were highly positively correlated with each other ($r = .60$, $p \leq .001$). The size of these correlations suggests that a relatively consistent CAR pattern should emerge for these three factors.

5.4. Influence of psychological factors and measurement day on CAR

As shown in Table 1, mixed-models yielded a significant effect of day ($p < .001$), a significant effect of psychological predictor (all $ps < .001$) and a significant day × psychological predictor interaction effect (all $ps < .01$). At first sight, our effects perfectly reflect the inconsistencies previously observed in the literature. First, despite the high intercorrelations mentioned above, the main effects of the psychological predictors are not consistent with each other. Specifically, *increased stress* is associated with *decreased* CAR ($F = 8.61$, $p < .001$), while *increased happiness* and *decreased neuroticism* are also associated with *decreased* CAR ($Fs = 3.20$ and 7.46 , $ps < .001$ respectively). Second, the

effect of one predictor on a given day contradicts the effect of the same predictor on another day (see Fig. 2).

While these contradictions perfectly fit with those observed in the literature, one very coherent and consistent interaction effect emerges across psychological predictors: the higher the psychological well-being (i.e., higher happiness, lower neuroticism, and lower perceived stress), the higher the difference between Sunday and Monday CARs. A mean split on our psychological predictors reveals that happy people display a significantly higher CAR on Monday than on Sunday ($t = -3.70$, $p < .01$), while unhappy people do not show any significant difference between Monday and Sunday CARs ($t = -0.44$, $p = ns$). Similarly, people reporting low stress display a significantly higher CAR on Monday than on Sunday ($t = -3.05$, $p < .01$), while people reporting high stress do not show any significant difference between Monday and Sunday CARs ($t = -0.67$, $p = ns$). Likewise, low neurotic individuals display a significantly higher CAR on Monday than on Sunday ($t = -3.44$, $p < .01$), while neurotic individuals do not show

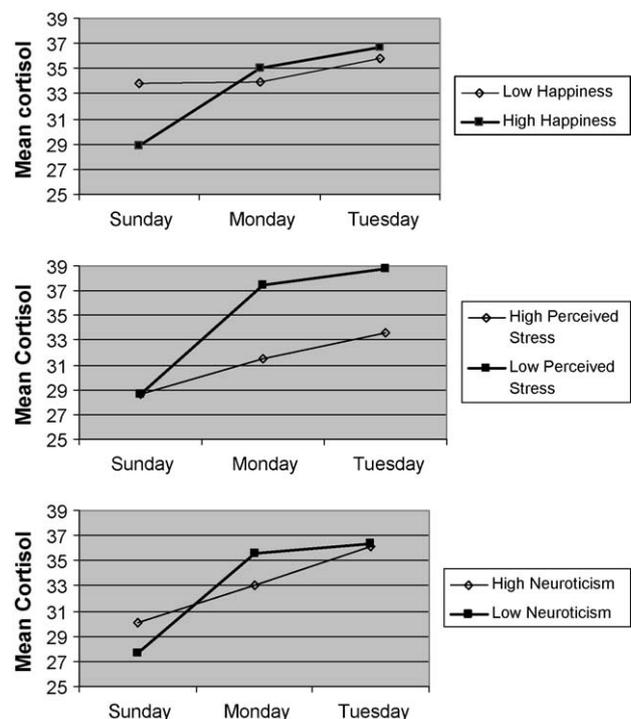


Fig. 2 Interactive effect of day and psychological factors on cortisol awakening response (expressed in nmol/l).

any significant difference between Sunday and Monday CARs ($t = -0.38$, $p = ns$).

6. Discussion

So far, findings in the awakening cortisol literature have been so contradictory that even the most scrupulous reviews (Fries et al., 2009) and comprehensive meta-analyses (Chida and Steptoe, 2009) could not make sense of all contradictions. Our study provides the first evidence that focusing on the CAR flexibility instead of the CAR magnitude might help resolving this puzzle.

Illustrating conflicting data that researchers have been facing over the last 10 years, the present study shows that focusing on the CAR magnitude led to highly inconsistent results. A small CAR was related to both increased stress and decreased neuroticism. The direction and effect size of the relationship between CAR magnitude and psychological variables also varied from one day to another. It seems therefore quite difficult to use the CAR magnitude to inform an individual's psychological condition. However, moving beyond CAR magnitude, focusing on CAR flexibility led to a way more coherent results pattern. All well-being indicators were systematically associated with a flexible CAR (i.e., varying according to environmental demands), whereas low well-being was associated with a stiff CAR (i.e., of equal intensity no matter the demands of the environment). Specifically, happier, less stressed, and less neurotic participants showed a more flexible CAR, with increased cortisol levels on week days and decreased levels during the week-end. By contrast, the more participants were unhappy, stressed and neurotic, the less their CAR varied between weekdays and weekends.

These results are in line with an increasing body of evidence showing that psychological factors show less consistent relationships with the absolute values of any given biological parameters than with the difference between two measurements of the given parameter on *different* occasions (Davydov et al., *in press*). For instance, positive mood is less related to the absolute blood pressure than to the difference between blood pressure measured in standing and supine positions (Davydov and Ritchie, 2009). This work that highlights the importance of flexibility in biological processes dovetails with numerous researches showing the importance of flexibility for psychological processes. For instance, a study on emotional processes showed that adjustment to the first 2 years of college depended less on regulatory strategies per se than on the ability to flexibly use different regulatory strategies in accord with situational demands (Bonanno et al., 2004). Our study suggests that the same mechanism applies to CAR and psychological well-being.

Although the cross-sectional design of the present study does not allow drawing conclusion regarding the direction of causality between our variables, one might hypothesize that the relationship between low well-being and a loss of flexibility in the CAR is bidirectional. First, it is possible that the disruption of the HPA axis is caused by psychological factors. Indeed, neuroticism, stress, and unhappiness are all associated with difficulties in regulating emotions which in turn is linked to exaggerated and/or chronic

cortisol secretion in response to stressors (Mikolajczak et al., 2007). Knowing that prolonged exposure to glucocorticoids damages the hippocampus (see Bremner, 1999 for a review) and that hippocampus seem to have a pivotal position in the regulation of the CAR (see Fries et al., 2009), it is possible that neuroticism, stress, and unhappiness lead to a less flexible CAR. However, the other causal arrow is also possible: a less flexible CAR could lead to higher neuroticism, stress, and unhappiness. Recent bio-behavioral research have shown that moods could be reliably predicted from the degree of concordance between biological activation and situational demand (Davydov et al., 2007). That is, negative moods occur when the level of neuro-endocrine or physiological arousal does not meet situational demands (i.e., is either too low or too high). In contrast, positive mood occurs when the level of arousal meets situational demands. Our results are perfectly in line with these findings. That is, a flexible adaptation of the CAR to situational demands was associated with positive states and traits (i.e., low stress, low neuroticism and high happiness). Conversely, a loss of flexibility—which resulted in a level of arousal that was either too low or too high compared to environmental demands—was associated with negative states and traits.

In short, the present study provides preliminary evidence that the notion of CAR flexibility may represent a fruitful new approach to start making sense out of the free cortisol response to awakening. Future studies that replicate the current results are needed before cortisol flexibility is used in the diagnosis and study of well-being. Those studies would benefit from using a larger sample than ours, and from controlling explicitly for protocol adherence (e.g., via the electronic monitoring of sampling times).

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Conflict of interest

None declared.

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