Assessing the Influence of the Inner Clock on the Cortisol Awakening Response and Pre-Awakening Movement

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***Abstract*—Our inner clock is responsible for creating a circadian rhythm that controls our sleep-wake cycle, and, hence, is also involved in the process of awakening from sleep. Awakening is accompanied by the cortisol awakening response (CAR). The purpose of the CAR is, presumably, to prepare our body for the upcoming challenges of the day. It is assumed that our inner clock is anticipating awakening, and thus, initiates the awakening process while we are still asleep, leading to the common phenomenon of waking up immediately before a known alarm. However, the role of the inner clock on the awakening process was only assessed in sleep laboratories and using invasive, blood-based biomarkers. For that reason, we investigated n = 117 participants by collecting cortisol data from saliva samples and IMU data from a wrist-worn inertial measurement unit (IMU) sensor node in a home environment over two nights. We compared cortisol data, characterizing the CAR, and IMU features, characterizing pre-awakening movement, between spontaneous awakening, awakening by a known alarm, and by an unknown alarm. We observed significant differences between the three study conditions in both cortisol and IMU data indicating higher cortisol reactivity and less movement if participants woke up by an unknown alarm. Our findings all support the assumption that our inner clock is anticipating our wake-up time. Utilizing our results, this work lays the foundation for the development of automatic classification models aimed at determining the ideal awakening time of individuals based on the analysis of pre-awakening movement.**

I. Introduction

On average, human adults spend 7-9 hours a day sleeping [1]. During workdays, our sleep is usually terminated by forced awakening via an alarm clock. However, some people tend to regularly wake up shortly before the alarm clock would ring [2]. It is assumed that our inner clock, which generates a circadian rhythm for physiological functions like heart rate, body temperature, and hormone secretion, is able to adapt to regular awakening times, and, thus, initiates the awakening process while we are still asleep.

The awakening process of humans is accompanied by the cortisol awakening response (CAR), a strong increase in cortisol levels atop the circadian rhythm in the first 30-45 min after awakening in the morning [3]. Previous work has linked the CAR to a "booting" function, preparing our body for the upcoming challenges of the next day [4]. Hence, assessing the CAR has been of great interest since it is a reliable marker for adrenocortical activity and has been linked with several psychosocial, physical, and mental health variables, such as socioeconomic status [5], (chronic) stress and burnout [6], and sleep quality [7].

The role of the inner clock on the CAR has been investigated by Kudielka et al. [8] who discovered that morning chronotypes ("early birds") show higher cortisol levels in the first hour after awakening than evening chronotypes ("night owls"). Stalder et al. [9] reported that the awakening mode (spontaneous awakening vs. forced awakening by an alarm) does not have an impact on the CAR. However, they only assessed differences between spontaneous awakening and a *known* alarm. Since the inner clock adapts to regular awakening times the actual "booting" effect of the CAR in the morning might not have been fully assessed. Thus, investigating the CAR after an *unknown* alarm might help to better understand the role of the inner clock on awakening.

The only known study to investigate the influence of an unknown alarm was published by Born et al. [2] who measured adrenocorticotropin and cortisol levels during and after sleep. Their results indicate increasing hormone levels already before a known awakening time but not for surprised awakening three hours earlier. However, the limited sample size ($n = 15$) and the rather obtrusive study procedure – participants spent three nights in a sleep laboratory while blood samples were regularly drawn – limits the transferability to real-world sleep scenarios.

For that reason, and to further investigate the influence of the inner clock on the awakening process, we extend the findings of existing work by comparing the CAR between spontaneous awakening and awakening by a *known* and an *unknown* alarm in a home environment. We complement this by additionally analyzing pre-awakening movement, assessed using a wrist-worn IMU sensor, since it was shown that movement is related to the current sleep stage [10] and could, therefore, also be an indicator for the initiation of the awakening process. To the best of our knowledge, our work is the first to assess the influence of the inner clock on pre-awakening movement and to assess the effect of an unknown alarm on the CAR in a home setting.

II. Methods

A. Data Acquisition

We acquired data from $n = 117$ healthy participants aged 24.2 ± 8.7 years (M $\pm$ SD) (79.5 % female) on two consecutive nights. All participants provided written informed consent before the study. To record movement during sleep and to objectively assess sleep and wake onsets all participants wore a 6-axis IMU sensor (Portabiles GmbH, Erlangen, Germany) on their non-dominant wrist during their whole time in bed.

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Sensor data were logged onto the internal storage with a sampling frequency of $102.4\,\mathrm{Hz}$ and were afterwards transmitted to a computer for subsequent data processing.

In the morning, five saliva samples were collected using Salivettes (Sarstedt AG & Co. KG, Nümbrecht, Germany) by chewing on a polystyrol roll for one minute. Participants were instructed to take the first saliva sample (S0) as soon as they woke up ($t = 0\,\mathrm{min}$ after awakening), followed by four other samples (S1-S4, $t = [15, 30, 45, 60]\,\mathrm{min}$ after S0). During that time, they were asked to fill out a survey to record saliva sampling times as well as self-assessed sleep onset and wake onsets. After collection, saliva samples were stored at $-18\,^{\circ}\mathrm{C}$ for later analysis.

We defined three different conditions that participants were randomly assigned to: In the *Spontaneous Awakening* condition they were instructed to wake up without setting an alarm. In the *Known Alarm* condition they were asked to set an alarm to their regular awakening time using an Android application we developed for this study[1]. In the *Unknown Alarm* condition participant were also instructed to set an alarm to their regular awakening time. However, when assigned to this condition, the smartphone application set a hidden alarm that woke them 21 min earlier in the first morning and 39 min in the second morning to prevent anticipation of the alarm in the second night. If participants were assigned to one of the two alarm conditions but woke up before the alarm, they were retrospectively reassigned to the *Spontaneous Awakening* condition for that night.

B. Data Processing

1) Cortisol Data: Salivary cortisol concentrations were determined in duplicate using a chemiluminescence immunoassay (CLIA, IBL, Hamburg, Germany) as described in previous publications (e.g., [11], [12]) after centrifuging the collected saliva samples at $2000\,\mathrm{g}$ and $20\,^{\circ}\mathrm{C}$ for five minutes. Apart from the raw cortisol values we computed features characterizing the CAR, such as the Area Under the Curve with respect to ground AUC_G, serving as measure for the total amount of cortisol secreted over time, and with respect to increase AUC_I, which is related to the sensitivity of cortisol secretion to changes over time [13]. As measures for the cortisol increase after awakening we additionally computed the maximum increase Δc_{max}, as well as the slope between S0 and S3 a_{S0S3}.

2) IMU Data: IMU data were used to objectively determine *sleep onset* and *wake onset* as well as features characterizing pre-awakening movement. For computing sleep and wake onset, IMU data was first processed to extract the time in bed using an algorithm from [14]. Sleep and wake phases were then determined from the resulting data using an algorithm by Cole et al. [15]. Since this algorithm was developed for activity counts as input data, we converted IMU data into activity counts according to [16]. Finally, we determined sleep onset as the beginning of the first sleep phase and wake onset as the end of the last sleep phase.

To characterize pre-awakening movement we extracted static periods from IMU data by first computing the L_2-norm of the gyroscope signal and then computing the variance of the norm in a window. We chose a window size of five seconds with 90 % window overlap and considered windows with a variance threshold of $\theta < 100\,m^2 s^{-4}$ as *static*. We then merged consecutive static windows to static periods and computed features based on all static periods with a duration $t \geq 60\,s$ within a 30 min interval before wake onset: number of static periods $|sp|$, duration of the longest static period sp_{max}, and the beginning of the longest static period $t(sp_{max})$. Additionally, we computed mean and standard deviation of the duration of static periods μ_{sp} and σ_{sp}.

C. Data Cleaning

For analysis we considered each of the two nights independently, resulting in a total of $n = 234$ samples. We filtered the collected cortisol data based on criteria from expert consensus guidelines for CAR assessment [3], which recommend excluding data where: (1) samples were missing (excluded in this step: $n = 8$), (2) wake onset or sampling times were missing ($n = 28$), (3) S0 was taken more than $5\,\mathrm{min}$ after wake onset (WO) ($\Delta t_{WO,S0} > 5$ min) ($n = 36$), (4) the time difference between two consecutive samples was too large $\left(|\Delta t_{S(i),S(i+1)} - 15\text{ min}| > 5\text{ min}\right)$ ($n = 5$), and (5) statistical outlier ($\geq 3\sigma$) occurred ($n = 15$).

From the collected IMU data we excluded (1) $n = 73$ samples due to missing or incomplete IMU recordings, mainly caused by participants' failure to adhere to the study protocol, and (2) $n = 30$ samples due to statistical outlier ($\geq 3\sigma$) in the IMU features. After data cleaning, $n = 142$ cortisol samples (*Spontaneous* vs. *Known Alarm* vs. *Unknown Alarm*: 69 vs. 37 vs. 36) and $n = 131$ IMU samples (*Spontaneous* vs. *Known Alarm* vs. *Unknown Alarm*: 61 vs. 42 vs. 28) remained for statistical analysis. All steps of data processing and cleaning were performed using *BioPsyKit*, our open-source Python library for the analysis of biopsychological data[2].

III. Statistical Analyses

For statistical analyses, we considered all our data to be normally distributed according to the central limit theorem as all conditions were $n \geq 30$ [17]. For the raw cortisol samples, we conducted mixed-measurement analyses of variance (ANOVA) to determine possible interaction effects between *sampling time* (within-factor) and *condition* (between-factor). For cortisol and IMU features, we used ANOVA, or Welch-ANOVA if homogeneity of variances, assessed by the Levene test, was violated, to determine group differences. As post-hoc tests we used pairwise t-tests in the case of Mixed-ANOVA and ANOVA, and pairwise Games-Howell post-hoc tests in the case of Welch-ANOVA, with Bonferroni correction for multiple-comparison correction. We set a significance level of $\alpha = 0.05$ and reported effect sizes as η_p^2 for (Welch-)ANOVA.

[1] https://play.google.com/store/apps/details?id=de.fau.cs.mad.carwatch

[2] https://github.com/mad-lab-fau/BioPsyKit

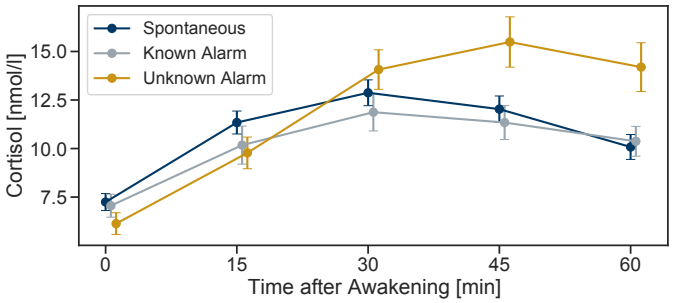

Fig. 1. Cortisol levels after awakening for different study conditions. Values are depicted as mean ± standard error.

In all Figures and Tables we used following notation to indicate statistical significance: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

IV. Results

1) Cortisol Data: Raw cortisol values of all participants are visualized per condition in Figure 1. It shows that, while all conditions show similar initial cortisol levels directly after awakening, the awakening mode seems to have an effect on the CAR, which is supported by a significant interaction effect ($F(8, 556) = 6.298, p < 0.001, \eta_p^2 = 0.083$). The *Spontaneous Awakening* and *Known Alarm* conditions reached their peak cortisol levels 30 min, the *Unknown Alarm* condition reached its peak 45 min after awakening. Maximum cortisol levels were considerably higher for the *Unknown Alarm* condition. After 45 min cortisol levels started to decline again for all conditions. Additionally, we observed significant group differences in the CAR features as shown in Table I and in Figure 2. Post-hoc testing revealed that the *Unknown Alarm* condition significantly differed from the other two conditions for AUC_I, Δc_{max}, and a_{S0S3}, while *Spontaneous Awakening* and *Known Alarm* appeared to be similar to each other.

TABLE I
ANOVA (BETWEEN-FACTOR: CONDITION) OF CORTISOL FEATURES.

	AUC_G	AUC_I	Δc_{max}	a_{S0S3}
F	1.211	4.178	5.452	6.876
p	0.301	0.017*	0.005**	0.001**
η_p^2	0.017	0.057	0.073	0.090

2) IMU Data: Welch-ANOVA of IMU features revealed significant differences in pre-awakening movement between the study conditions (Table II). Post-hoc testing confirmed that the longest static period within 30 min before awakening was significantly shorter in the *Unknown Alarm* condition compared to the *Spontaneous Awakening* condition and also occurred earlier. Additionally, pre-awakening movement showed a significantly higher standard deviation compared to the two alarm conditions indicating more movement before awakening without an alarm.

V. Discussion

The main objective of our study was to investigate the influence of the inner clock on the awakening process by comparing *Spontaneous Awakening* with awakening by a *Known Alarm* and an *Unknown Alarm*. Our results confirm previous findings that the CAR does not differ between spontaneous awakening and awakening by a known alarm [9]. However, awakening from an unknown alarm showed significant differences in both raw cortisol and cortisol features. While AUC_G did not differ, AUC_I, a_{S0S3}, and Δc_{max}, which serve as measures for cortisol reactivity after awakening, were significantly higher for the *Unknown Alarm* condition compared to the other two conditions. Furthermore, the cortisol maximum appeared later. This might indicate that the inner clock has already initiated the CAR during sleep in order to prepare for awakening and further underscores the "booting" function of the CAR.

Our findings are also partly visible in the features assessing pre-awakening movement. Participants that woke up without an external alarm experienced more movement before awakening, indicated by a significantly higher standard deviation of static period duration σ_{sp}. Additionally, the longest static period before awakening occurred later for the *Unknown Alarm*, thus indicating more movement since the duration of static periods decreased before awakening. Synthesizing our results, we found strong indications that the inner clock supports the booting process in the morning by anticipating the

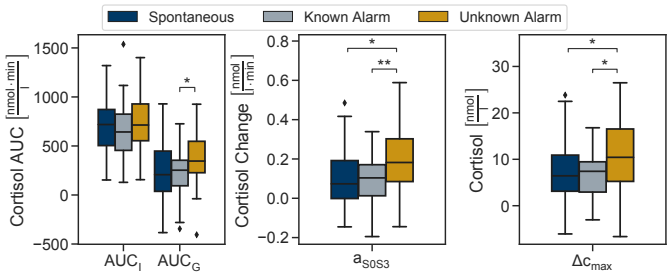

Fig. 2. Cortisol-derived features for different study conditions.

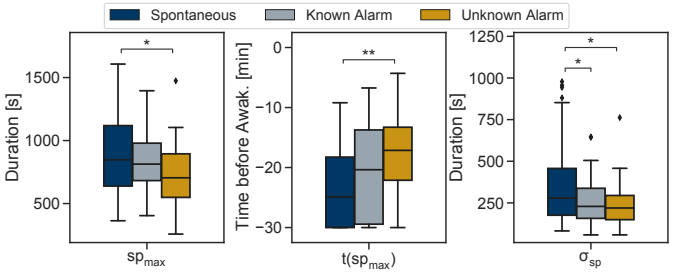

Fig. 3. IMU-derived features for different study conditions.

TABLE II
WELCH-ANOVA (BETWEEN-FACTOR: CONDITION) OF IMU-BASED FEATURES OF STATIC PERIODS WITHIN 30 MIN BEFORE AWAKENING.

	$\|sp\|$	sp_{max}	$t(sp_{max})$	μ_{sp}	σ_{sp}
F	3.119	4.219	6.644	5.027	5.607
p	0.051	0.018*	0.002**	0.009**	0.005**
η_p^2	0.047	0.061	0.090	0.057	0.086

awakening time and thus initiating awakening process while we are still asleep. This is supported by significant differences between the three study conditions in post-awakening cortisol levels as well as in pre-awakening movement.

However, there are also some limitations to our study which need to be addressed in future work. Our study participants were mostly young, healthy adults, and almost 80 % were female. Even though statistical testing did not show any significant gender differences, our findings need to be confirmed using a more representative study population. Due to missing participant compliance we had to exclude 33 % of cortisol data (cortisol exclusion criteria (1)-(4)) and 31 % of IMU data (IMU exclusion criteria (1)) in total. This is a common problem in unsupervised studies at home, especially for CAR assessment, since only small sampling time differences can significantly alter the CAR [3]. For that reason, increasing participant compliance, for example through the use of a mobile application that tracks sampling times, would be of great importance for future work.

It is further noteworthy that the maximum cortisol levels for the *Unknown Alarm* condition appeared not only later after awakening, but they were also, on average, higher while the initial cortisol levels after awakening did not differ. This can be a side effect from the awakening mode: Getting woken up by an unknown alarm might represent a more arousing event than "regular" awakening and could thus contribute to the increased cortisol peak. Some participants reported that they assumed an error in the application when the unknown alarm in the application went off before the actual alarm time until they realized the application was working correctly. Thus, we can not rule out that the unknown alarm had – to a limited degree – an enhancing effect on the CAR which is not solely explained by the effect of the inner clock. However, since we were also able to observe movement differences before awakening, this effect does, in our opinion, not pose a significant confounder.

VI. Conclusion and Outlook

In this work we investigated the role of the inner clock in triggering the awakening process. We collected saliva samples to assess the cortisol awakening response (CAR), and collected IMU data from a wrist-worn IMU sensor to assess pre-awakening movement. While our results confirmed the findings of previous work that the CAR does not differ between *Spontaneous Awakening* and forced awakening by a *Known Alarm*, our third condition, forced awakening by an *Unknown Alarm*, showed significant differences in the CAR, indicating higher HPA axis reactivity. This is supported by less movement for the *Unknown Alarm* condition, characterized, for instance, by a significantly lower standard deviation of static periods in the last 30 min before awakening, and the longest static period occurring closer to awakening.

Our findings all support the assumption that our inner clock is anticipating our wake-up time and is thus already initiating the awakening process while we are still asleep by increasing cortisol activity. Besides the aforementioned improvements to the study design to increase participant compliance future work should perform a more detailed analysis by including psychological trait and state variables that might influence the CAR into statistical models. However, with our work, we lay the foundation for future work, which can use our findings to develop automatic classification models to determine the ideal awakening time of individuals based on pre-awakening movement by using the CAR as ground truth.

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