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Overnight heart rate variability and next day cortisol response during simulated on-call conditions

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ABSTRACT

Objective: This study had two specific objectives, 1) to investigate the impact of being on-call on overnight heart rate variability during sleep and; 2) to examine whether being on-call overnight impacted next-day salivary cortisol concentrations.

Methods: Data are reported from three within-subject laboratory studies (n = 24 in each study) that assessed varying on-call conditions. Healthy male participants (n = 72 total) completed a four-night laboratory protocol, comprising an adaptation night, a *control* night, and two counterbalanced on-call nights with varying on-call conditions. These on-call conditions were designed to determine the impact of, Study 1: the likelihood of receiving a call (*definitely, maybe)*, Study 2: task stress (*high-stress, low-stress)*, and Study 3: chance of missing the alarm (*high-chance, low-chance)*, on measures of physiological stress. Overnight heart rate variability (HRV) (during sleep) was measured using two-lead electrocardiography, and time- and frequency-domain variables were analysed. Saliva samples were collected at 15-min time intervals from 0700-0800 h to determine cortisol awakening response outcomes and at four daily time points (0930 h, 1230 h, 1430 h, and 1730 h) to assess diurnal cortisol profiles.

Results: There were few differences in HRV measures during sleep across all three studies. The only exception was in Study 1 where the standard deviation of the time interval between consecutive heartbeats and the root mean square of consecutive differences between heartbeats were lower across all sleep stages in the *definitely* condition, when compared to *control*. Across all three studies, being on-call overnight also had little impact on next-day cortisol awakening response (CAR), with the exception of Study 2 where the 1) CAR area under the curve with respect to increase was blunted in the *high-stress* condition, compared to the *control* and *low-stress* conditions and, 2) CAR reactivity was higher in *low-stress* condition, compared with the *high-stress* condition. In Study 1, diurnal cortisol area under the curve with respect to ground was lower in the on-call conditions (*definitely* and *maybe*) when compared to control. There were no differences in diurnal cortisol measures in Study 3.

Conclusion: This is the first study to investigate how different aspects of being on-call affect physiological stress responses. Overall, relatively little differences in measures of overnight heart rate variability and next-day cortisol response were recorded in all three studies. Further research utilising real on-call work tasks, not just on-call expectations (as in the current study) will help determine the impact of on-call work on the physiological stress response.

1. Introduction

On-call or stand-by work is an occupational arrangement where an employee must be available to be contacted to start, or resume work, at short notice (Australian Bureau of Statistics, 2016; Bamberg et al., 2012). Over the past decade, irregular work schedules have become increasingly prevalent worldwide (Golden, 2015; Parent-Thirion et al., 2012). Significant percentages of the Australian (25%;) (Australian Bureau of Statistics, 2012), European (10–20%) (Burri et al., 2018; Parent-Thirion et al., 2012), and United States (2–17%) (Katz and Krueger, 2016; Labor, 2005) workforces are involved in on-call work arrangements. On-call work typically benefits organisations by allowing flexibility (i.e., the ability to respond to changes in demand and other contingencies) without the financial cost of full shift coverage on-site

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(McCrate, 2018; Nicol and Botterill, 2004). However, on-call work is not always beneficial to the worker, as personnel report impaired sleep (Ferguson et al., 2016; Paterson et al., 2016; Vincent et al., 2018a), increased work-family conflict (Jay et al., 2018; Lindfors et al., 2006), compromised social life (Imbernon et al., 1993), and increased stress levels (Dettmers et al., 2016). Heightened physiological stress is known to adversely impact worker health (Chandola et al., 2006), as well as increase the risk of chronic disease (e.g., cardiovascular disease, Type 2 diabetes) (Chrousos, 2009; Rosmond and Björntorp, 2000). Hence, understanding the physiological stress response to on-call work is critical to support worker health and well-being, job satisfaction, and turnover rates (Heponiemi et al., 2008).

The stress associated with on-call work has received recent attention (Hall et al., 2017a, b; Ziebertz et al., 2015), and is thought to result from factors including the inherent unpredictability of calls, rumination about the actual work to be performed when called, and the inability of workers to 'switch off' (Bamberg et al., 2012; Paterson et al., 2016). Previous research suggests there is a bi-directional relationship between sleep and physiological stress (Steiger, 2002). This is important to consider given that on-call work is associated with impairments to sleep (Ferguson et al., 2016), even when no calls are received (Torsvall and Akerstedt, 1988; van de Ven et al., 2015). Research highlighting on-call work as an occupational stressor is largely based on self-report measures of stress (Ziebertz et al., 2015). For example, doctors have reported on-call work as a major source of stress and job dissatisfaction (Dowell et al., 2000; Lindfors et al., 2006). Research also shows on-call firefighters report being 'worried their pager was broken' after multiple nights of few, or no calls, indicating that stress responses on-call may be present regardless of whether the individual is required to report to work (Paterson et al., 2016). However, self-report measures of stress are not always consistent with physiological measures (Harbeck et al., 2015; Simpson et al., 2016). For example, in response to a 24-h on-call shift, physicians self-reported increased stress without any corresponding increases in physiological measures of stress such as heart rate variability (HRV) or cortisol (Harbeck et al., 2015).

Studies investigating the impact of on-call work on physiological measures of stress are limited and the findings are equivocal. HRV, or the beat-to-beat change in heart rate, is a non-invasive indicator of autonomic nervous system activity (Berntson et al., 1997). Several investigations have noted changes in waking HRV measures in response to real-world on-call work, with some (Amirian et al., 2014; Malmberg et al., 2011; Rauchenzauner et al., 2009; Tobaldini et al., 2013a) but not all (Kikuchi et al., 2018; Thurman et al., 2017) indicating increased physiological stress during on-call work itself. Furthermore, another study reported that when anticipating a stressful task (albeit not on-call), changes in HRV during sleep were observed (Hall et al., 2004). However, no research to date has examined the impact of anticipatory stress preceding a period of on-call work on measures of HRV during sleep. During normal sleep, HRV is influenced by the stage of sleep, for example, rapid eye movement sleep (REM) is characterised by sympathetic predominance and vagal withdrawal, while the opposite is observed during non-rapid eye movement (NREM) sleep (Tobaldini et al., 2013b). Therefore, research is needed to examine the impact that on-call conditions may have on HRV during sleep, while also considering sleep stage.

Cortisol is the most widely accepted biomarker of hypothalamo-pituitary adrenal axis activation (Kirschbaum and Hellhammer, 1989). Some on-call studies have reported no difference in workers' evening salivary cortisol concentrations (Bamberg et al., 2012), diurnal cortisol levels (Hall et al., 2019), 24-h urinary cortisol (Ernst et al., 2014), and 24-h salivary cortisol (Malmberg et al., 2007). In contrast, other research has demonstrated on-call work is associated with alterations in the functioning of the physiological stress systems, showing a steeper increase in the area under the curve with respect to increase (AUC_I) of salivary cortisol in response to waking (Dettmers et al., 2016), increased 24-h urinary excretion of adrenaline (Samel et al., 2004), and noradrenaline (Ernst et al., 2014; Samel et al., 2004). Blunted cortisol awakening response (CAR) peak, and post-awakening cortisol AUC_G response (Hall et al., 2019) have also been noted under on-call conditions. Contradictory findings from previous studies are likely the result of differences in research protocols, for example, the on-call conditions themselves (e.g., likelihood of being called, task performed upon waking), use of different self-report and/or physiological stress measures, and sample timing. Well-controlled laboratory studies and standardised measurement techniques (Stalder et al., 2016) may assist to isolate the precise components of on-call work contributing to the physiological stress response.

From existing on-call literature (Ferguson et al., 2016; Hall et al., 2017b), we identified and isolated three factors that could potentially contribute to increased physiological stress when on-call: a) the likelihood of receiving a call; b) the stressfulness of the task performed when called; and c) the possibility of missing a call. From existing on-call literature, we identified and isolated three factors that could potentially contribute to increased physiological stress when on-call: a) the likelihood of receiving a call; b) the stressfulness of the task performed when called; and c) the possibility of missing a call (Ferguson et al., 2016; Hall et al., 2017b). Previous research has reported that the likelihood of receiving a call can differentially impact sleep behaviour when on-call (Jay et al., 2016; Wuyts et al., 2012). For example, when participants were told they 'could' be called during a night on-call there were significant differences in sleep outcomes (e.g., longer sleep onset latency, more wake after sleep onset), compared to a night not on-call (Wuyts et al., 2012). Conversely, another study found no differences on on-call and not-on call sleep outcomes, when participants were told they would 'definitely' be called on the on-call nights (Jay et al., 2016). Prior studies also indicate that the importance of the task required upon waking following a call may influence anxiety (Åkerstedt, 2006), apprehension (Kecklund and Åkerstedt, 2004) and task performance (Sprajcer et al., 2018a). Finally, in a qualitative study on-call workers who did not receive calls following consecutive on-call nights reported they were 'worried about missing calls' and this adversely impacted their sleep (Paterson et al., 2016). However, the relative contribution of each of these on-call factors (likelihood of receiving a call, stressfulness of the task performed, and possibility of missing a call) Vincent et al., 2018b; Larsen et al., 2015. The relative contribution of each of these factors on workers' physiological stress is unknown, as multiple external confounding variables are usually present including, but not limited to, the amount of prior sleep, timing of calls, noise exposure, and physical activity levels.

To minimise the effect of extraneous variables, three controlled laboratory studies were conducted. Each study examined the relative impact of on-call factors on two measures of physiological stress (i.e., HRV and salivary cortisol concentrations). The aim of this study was to investigate the impact of different on-call factors, namely call likelihood, stressfulness of the task performed when called, possibility of missing a call on, 1) overnight HRV during sleep and; 2) next-day salivary cortisol concentrations. It was hypothesised that on-call nights when on-call demand was greatest (e.g., definitely receiving a call, high-stress task performed upon waking and high-chance of missing a call) would be associated with a) would be associated with reduced HRV measures related to stress and autonomic nervous activity during sleep b) increased next-day salivary cortisol concentrations when compared to on-call nights with less demand (maybe receiving a call, low-stress task performed upon waking and low-chance of missing a call) and nights not on-call.

2. Methods

2.1. Participants

Healthy male adults (n = 72) were recruited in Adelaide, Australia. Participant characteristics are reported in Table 1. Participation was voluntary and ethical approval was obtained from the Human Research Ethics Committee of Central Queensland University (H15/07-158). Participants provided written consent and were remunerated financially for their time (AU$480).

Table 1
Participant demographics.

	Study 1 (n = 24)	Study 2 (n = 23)	Study 3 (n = 24)
Age (y)	28.0 ± 6.0	27.0 ± 4.0	25.0 ± 4.0
Body mass (kg)	76.5 ± 8.6	74.6 ± 10.6	74.5 ± 11.4
Height (cm)	180.2 ± 7.4	176.9 ± 6.0	177.4 ± 7.2
Body mass index ($kg \cdot m^2$)	23.3 ± 1.9	23.7 ± 2.8	23.6 ± 3.0
Pittsburgh Sleep Quality Index	2.5 ± 1.3	2.5 ± 1.2	2.5 ± 1.3
Epworth Sleepiness Score	4.0 ± 2.2	3.6 ± 2.2	3.9 ± 2.4

Data are presented as mean ± SD.

A general health questionnaire was used to screen participants to determine whether they were eligible to participate in the study. The inclusion criteria were: aged between 20–35 years; non-shiftworker; non-smoker; consumption of ≤ 10 standard alcoholic beverages/week; caffeinated beverage consumption equal to or less than 120 mg/day (˜2 cups of coffee); habitual bedtimes between 2200-0000 h; rise times between 0600-0800 h; no history of habitual napping; no previous diagnosis of psychiatric and/or neurological problems; no trans-meridian travel in the previous four weeks; free from medication and drugs acting on the central nervous system known to interfere with sleep or cortisol; no glucose- and/or lipid-lowering medication. Participants were also required to have a BMI < 30 $kg \cdot m^2$, a global Pittsburgh Sleep Quality Index ≤ 5 (Buysse et al., 1989), an Epworth Sleepiness Scale score < 10 (Johns, 1991), normal or mild scores on the 42-item Depression Anxiety Stress Scale (Lovibond and Lovibond, 1995), and moderately morning/evening or neither chronotype, on the Horne-Ostberg Morningness/Eveningness Questionnaire (Horne and Ostberg, 1975).

2.2. Study design

The data reported here are from three within-subject studies (n = 24 in each study). Participants lived in a time-isolated, sound-attenuated and temperature-controlled (21 ± 2 °C) laboratory for five days and four nights. The protocol consisted of an adaptation night, a control night, and two counterbalanced 'on-call' nights. The adaptation and control days were the same between studies, but the 'on-call' days (On-call 1 and On-call 2) were different depending on the conditions (i.e., condition A or condition B) for each study (see Fig. 1). The order of the on-call conditions were counterbalanced (i.e., 12 participants in each study completed condition A followed by condition B and 12 participants completed condition B followed by condition A).

2.3. Pre-experimental procedures

Following initial screening, participants attended a familiarisation visit at the research laboratory and were provided the opportunity to ask questions about the experimental protocol. In the week prior to entering the study, participants were instructed to maintain their normal sleep routine to minimise any potential carry-over effects of sleep loss. Participants were also required to refrain from engaging in moderate and/or vigorous physical activity for at least 48 h prior to commencing the study. To ensure compliance to the habitual sleep routine and physical activity requirements, participants wore an activity monitor (Actical MiniMitter/Respironics, Bend, OR) on their non-dominant wrist and completed a sleep diary for the week leading up to each study Vincent et al., 2016a; Vincent et al., 2016b.

2.4. Experimental procedure

2.4.1. Arrival day, adaptation night and adaptation day

Participants arrived at the laboratory at 1700 h on the adaptation night, where they completed cognitive task familiarisation and were instructed on daily procedures. Prior to bedtime, participants were informed that they would receive a full uninterrupted night's sleep and be woken at the appropriate time in the morning (see Table 2). The following day, participants were further trained on the cognitive performance tasks to minimise learning effects.

2.4.2. Daily procedure

Each night at 2000 h a standardised montage of electrodes was applied to the head and face of each participant in preparation for HRV measurement (for further detail see *Measures*), overnight sleep (polysomnography) measurement and sleep scoring (published elsewhere; (Sprajcer et al., 2018c)). At 2255 h every night the electrodes were connected to the recording hardware, before lights out at 2300 h. The sleep opportunity for each night was from 2300-0700 h (8 h), including on-call nights. On the experimental days (control, on-call condition A, and on-call condition B), cognitive performance and salivary cortisol were measured at 0700 h, 0715 h, 0730 h, 0745 h, and 0800 h, in order to capture the CAR and the effects of sleep inertia on performance (to be published elsewhere). Cognitive test batteries were performed and salivary cortisol were taken at 0930 h, 1230 h, 1430 h, and 1700 h, on each of these days to identify participants' diurnal profiles. Further details regarding salivary cortisol collection are provided in *Measures*. For all days, when participants were not completing testing, they were allowed to partake in quiet activities (e.g. read books or watch television) in their own rooms and ate their daily meals in a communal dining room.

2.4.3. Control night and control day

On the control night prior to bedtime participants were informed that they were not on-call (see Table 2). The next day, participants were woken by overhead lights and a knock at their bedroom door.

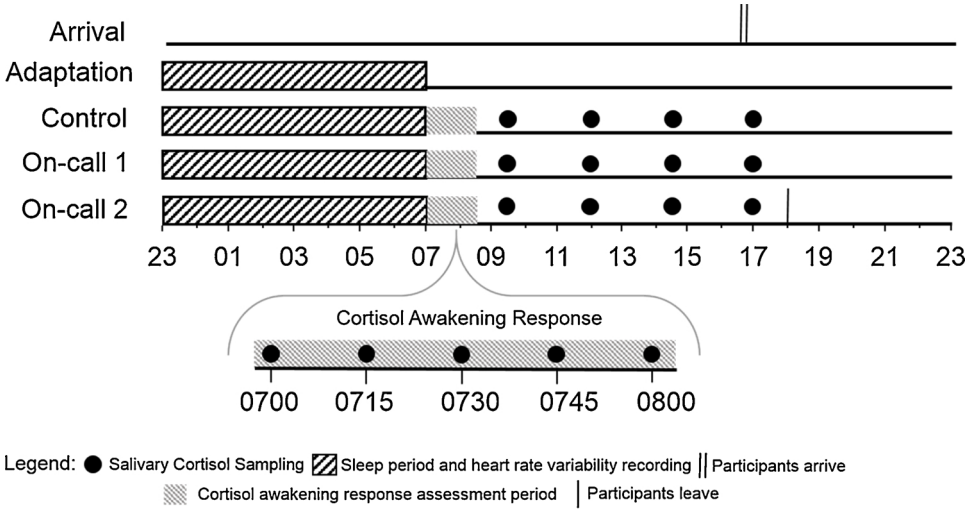
2.4.4. On-call nights and days

On the third and fourth nights in the laboratory, participants were told that they were on-call. On these nights, prior to bed, participants were given instructions about being on-call, with descriptions varying depending on which study they were in, 1) likelihood of receiving a call, 2) task stress or 3) chance of missing a call (see Table 2). On-call instructions were delivered prior to bed at 2230 h. To ensure that daytime cortisol levels were not affected by the expectation of a call, participants were only on-call overnight. On both on-call days in Study 1 and Study 2, participants were woken by an alarm (TOA Transistor megaphone, model: ER-1215S, 61.5–69.8 dB (A)) at 0700 h and immediately completed the sleep inertia testing battery on awakening. In Study 3, participants were also woken by an alarm in the *low-chance* condition, and in the *high-chance* condition, were woken by a researcher (see Table 2 for more information). In Study 2, following the sleep inertia battery, participants completed an additional task, which was to either perform a 5-min speech (Condition A) or to read quietly (Condition B). Participants then completed the daytime testing and saliva collection.

2.5. Measures

Sleep

During each sleep period, polysomnographic recordings were obtained (Bloch, 1997) and used to examine the impact of experimental conditions on sleep macro-architecture derived from traditional sleep scoring. Electrodes were used in a standard configuration, with electroencephalographic (EEG), electromyographic (EMG) and electro-oculographic (EOG) recordings taken for each participant. C3/M2, F4/M1 and O2/M1 channels were used, and a trained sleep technician scored each sleep period in 30-s epochs according to standard criteria (Iber, 2007). Variables generated include total sleep time (TST), sleep onset latency, wake after sleep onset, sleep efficiency ((TST/time in bed)*100), latency to 10 min of sleep, latency to N3, REM (rapid eye movement sleep) latency, minutes and proportion of total sleep time for



Study	On-call factor	On-call conditions (A or B)
1	Likelihood of receiving a call	*definitely or maybe*
2	Task stress	*high-stress or low-stress*
3	Chance of missing a call	*high-chance or low-chance*

Fig. 1. Protocol, study design and conditions.

each sleep stage (N1, N2, N3, REM, NREM (non-REM sleep)), stage shifts, awakenings and arousals in each sleep stage.

Heart rate variability

2.6. Measurement of ECG

HRV is widely used as a non-invasive and reliable tool to evaluate cardiovascular autonomic control in health and disease (Tobaldini et al., 2013b). HRV was measured during the night-time sleep period using two electrocardiography (ECG) electrodes (left-positive and right-negative) and recorded using the Grael PSG/EEG system (Compumedics, Melbourne, Victoria, Australia). At 2000 h, two ECG electrodes were applied, one negative ECG electrode was placed 3 cm below the right clavicle, and was positioned on the torso parallel to the right leg. The positive ECG electrode was positioned on the left side of the torso parallel to the left hip and leg, between either the fifth, sixth, or seventh intercostal spaces on the lower left hand side of the rib cage.

2.7. Analysis of heart rate variability

The QRS complex (i.e, the three graphical deflections seen on a typical electrocardiogram) were detected from ECG using the filter bank algorithm (Afonso et al., 1999). For sleep stage-specific HRV analysis, RR interval (i.e., the time interval between consecutive heart beats) time series were extracted for all 5-min intervals of constant sleep stage. HRV outcome variables were computed for all 5-min segments and median values calculated for further statistical analysis. For power spectrum analysis, RR interval time series were linearly interpolated at 4 Hz and subjected to a fast Fourier transform algorithm combined with a Blackman-Harris window.

2.8. Heart rate variability outcome variables

The following HRV outcome variables were evaluated in this study, content adapted using the following resources (Chouchou and Desseilles, 2014; European Society of Cardiology, 1996): mean NN (milliseconds; ms) – the average heart period; SDNN (ms) – standard deviation of the time interval between normal beats; RMSSD (ms) – the root mean square of consecutive differences between normal heartbeats (European Society of Cardiology, 1996); LF (log ms^2) – logarithmic function of the absolute power of heart rate oscillations in the low-frequency band (0.04-0.15 Hz) (European Society of Cardiology, 1996); HF (log ms^2) – logarithmic function of the absolute power of heart rate oscillations in the high-frequency band (0.15-0.4 Hz) (European Society of Cardiology, 1996). Values were expressed in either original units or as the natural logarithm of original units to normalise the distribution, consistent with previous research methodologies on heart rate variability (European Society of Cardiology, 1996).

Salivary cortisol

2.9. Saliva collection for cortisol sampling

Cortisol is reliably measured in saliva (Elder et al., 2014). Salivary cortisol samples were collected using a cotton swab (Salivette, Sarstedt, Nurnbrect, Germany). As per the manufacturer instructions, participants were asked to roll the cotton swab in their mouth for 2 min. To prevent sample contamination from food or fluid intake, participants were instructed to refrain from eating or drinking 10 min prior to saliva collection. Samples were centrifuged for 10 min at 3000 × g and then stored at ≤ -80 °C until analysis.

2.10. Salivary cortisol analyses

Salivary cortisol concentrations were determined using high sensitivity (0.005 μg/dL) enzyme-linked immunosorbent assay (ELISA) kits (IBL International, Hamburg, Germany). Samples were analysed in duplicate and each participant was analysed in a single assay in order to reduce error of variance. To determine the concentration of cortisol in each sample, absorbance was determined using a Synergy 2 microplate reader (BioTek Instruments, Inc., Vermont) and Gen5 Software (BioTek Instruments, Inc., Vermont), and compared to a standard curve. In accordance with the manufacturer's instructions, the following correction factors were applied to the Study 2 and Study 3 data, respectively (y = -0.009988 + 0.645578x) and (y = -0.003142 + 0.616143x).

For Study 1, the intra-assay coefficients of variation were, 8.1% at 2.0 $nmol{\cdot}L^{-1}$, 9.4% at 7.2 nmol·L-1, and 7.2% at 15.8 $nmol{\cdot}L^{-1}$; the inter-assay coefficients of variation were 8.0% at 2.1 $nmol{\cdot}L^{-1}$, 12.4% at 5.3 $nmol{\cdot}L^{-1}$ and 6.3% at 16.8 $nmol{\cdot}L^{-1}$. For Study 2, the intra-assay coefficients of variation were 4.3% at 2.1 $nmol{\cdot}L^{-1}$, 3.7% at 2.9 $nmol{\cdot}L^{-1}$, and 8.0% at 10.6 $nmol{\cdot}L^{-1}$; the inter-assay coefficients of variation were 9.7% at 1.9 $nmol{\cdot}L^{-1}$, 13.2% at 3.0 $nmol{\cdot}L^{-1}$, and 12.4% at 11.4 $nmol{\cdot}L^{-1}$. For Study 3, the intra-assay coefficients of variation

Table 2
On-call instructions for each study.

Study	Condition	On-call Instructions	On-call Protocol
All	Adaptation	"You are not on-call tonight. In the morning, a researcher will come into your room, turn the lights on low and wake you up at the right time"	Lights come on in participant rooms, researchers enter their rooms to wake them up at 0700 h.
	Control	"You are not on-call tonight. In the morning, a researcher will come into your room, and wake you up at the right time"	Lights come on in participant rooms, researchers enter their rooms to wake them up at 0700 h. Saliva sampling for CAR and sleep inertia testing begins.
1: Likelihood of receiving a call	*Definitely*	"Tonight you are on-call and **you will definitely get called during the night**. As soon as you hear the sound you must press your call button as quickly as possible so we know that you have heard it. The call signal is the very loud alarm that we demonstrated earlier in the study. You will definitely be woken by the sound; no participant has ever slept through it. Once you have pressed the button, stay lying in bed with your eyes open until a researcher gives you your next instructions. Following the call you will complete some tests. You will definitely be called at some stage during the night."	Loud alarm sounded at 0700 h, followed by lights on and a researcher entering participant rooms. Saliva sampling for CAR and sleep inertia testing begins.
	Maybe	"Tonight you are on-call and **you may or may not get called during the night**. As soon as you hear the sound you must press your intercom button as quickly as possible so we know that you have heard it. The call signal is the very loud alarm that we demonstrated earlier in the study. You will definitely be woken by the sound; no participant has ever slept through it. Once you have pressed the button, stay lying in bed with your eyes open until a researcher gives you your next instructions. Following the call you will complete some tests. You may, or may not get called during the night"	Loud alarm sounded at 0700 h, followed by lights on and a researcher entering participant rooms. Saliva sampling for CAR and sleep inertia testing begins.
2: Task stress	*High- stress*	"Tonight you are on-call and you will definitely get called during the night. As soon as you hear the sound, you must press the button beside your bed as quickly as possible, so we know that you have heard it. The call signal is a very loud alarm. You will definitely be woken by the sound; no participant has ever slept through it. Once you have pressed the call button, stay lying in bed with your eyes open until a researcher gives you your next instructions. Following the call, you will complete some tests. **After these tests you will be asked to perform a speech in front of a researcher.** The speech will be recorded by the cameras in your room and the researchers listening will evaluate the speech for content and quality. You will be given the topic of your speech and 3 minutes to prepare your speech after the tests."	Loud alarm sounded at 0700 h, followed by lights on and a researcher entering participant rooms. Saliva sampling for CAR and sleep inertia testing begins. After sleep inertia testing, participants performed a speech in front of a researcher. They were given 3 min to prepare, and spoke (while standing) for 5 min.
	Low- stress	"Tonight you are on-call and you will definitely get called during the night. As soon as you hear the sound, you must press the call button beside your bed as quickly as possible, so we know you that have heard it. The call signal is a very loud alarm. You will definitely be woken by the sound; no participant has ever slept through it. Once you have pressed the button, stay lying in bed with your eyes open until a researchers gives you your next instructions. Following the call, you will complete some tests. **After these tests you will be asked to read a magazine to yourself for approximately 5 minutes**. No researchers will be present while you are reading, and you will not be asked anything about the articles you choose to read. We will provide you with a magazine."	Loud alarm sounded at 0700 h, followed by lights on and a researcher entering participant rooms. Saliva sampling for CAR and sleep inertia testing begins. After sleep inertia testing, participants read quietly for 5 min.
3: Chance of missing a call	*High- chance*	"Tonight you are on-call and you will definitely get called during the night. As soon are you hear the sound, you must press the call button beside your bed as quickly as possible, so we know that you have heard it. **The call signal is a soft alarm.** Participants have previously slept through it. It is very important that you press the button next to your bed as quickly as possible. Once you have pressed the button, stay lying in bed with your eyes open until a researcher gives you your next instructions. Following the call, you will complete some tests."	Lights on and a researcher entering participant rooms to wake them at 0700. Participants are all told that they missed the alarm, but no alarm was ever sounded. Saliva sampling for CAR and sleep inertia testing begins.
	Low- chance	"Tonight you are on-call and you will definitely get called during the night. As soon are you hear the sound, you must press the call button beside your bed as quickly as possible, so we know you that have heard it. **The call signal is a very loud alarm.** You will definitely be woken by the sound; no participant has ever slept through it. Once you have pressed the button, stay lying in bed with your eyes open until a researchers gives you your next instructions. Following the call, you will complete some tests."	Loud alarm sounded at 0700, followed by lights on and a researcher entering participant rooms. Saliva sampling for CAR and sleep inertia testing begins.

CAR (cortisol awakening response).

were 7.1% at 0.8 nmol·L^{-1}, 6.6% at 1.7 nmol·L^{-1} and 3.9% at 5.6 nmol·L^{-1}; the inter-assay coefficients of variations were 25.6% at 1.6 nmol·L^{-1}, 12.2% at 3.4 nmol·L^{-1}, and 19.8% at 10.8 nmol·L^{-1}. For all three studies, the intra-assay coefficient of variation was below the recommended 10% (Salimetrics, 2010). For Study 1 and Study 2, the inter-assay coefficient of variation was below the recommended 15% (Salimetrics, 2010). The inter-assay coefficient of variation for Study 3 was higher than the recommended 15% at two of three concentrations. The approach we applied to account for this high variation is explained in the *Statistical analyses* section below.

2.11. Salivary cortisol outcome variables

The CAR and diurnal cortisol profile were analysed. The CAR was assessed using samples collected at 0700 h, 0715 h, 0730 h, 0745 h and 0800 h. Thus, they were synchronised to both time of awakening (0700 h) and time of day. CAR area under the curve in respect to increase (AUC_I) and post-awakening area under the curve in respect to ground (AUC_G) were calculated for each using the trapezoidal method (Pruessner et al., 2003). AUC_G provides a measure of total cortisol output over the entire CAR period (Clow et al., 2004), while AUC_I provides a measure of the intensity of the change of cortisol concentrations. The cortisol concentration at time point 0700 h represented the CAR 'awakening' concentration, and the CAR 'peak concentration' was the highest concentration during the CAR period. The difference between the cortisol concentration upon waking (0700 h) and the highest CAR peak concentration was used to calculate CAR reactivity.

To determine the diurnal cortisol profile, the samples at (0700 h, 0930 h, 1200 h, 1430 h, 1700 h) were analysed. For each participant, diurnal cortisol AUC_G was calculated. AUC_G, calculated using the Equation 2 trapezoidal method (Pruessner et al., 2003), provides an estimate of average cortisol exposure (Adam and Kumari, 2009).

2.12. Statistical analyses

All statistical analyses were performed using R, and all models were fitted using the LmerTest package (version 3.0) (R Development Core Team, 2008). To investigate the impact of the conditions for each study, separate linear mixed-effects models were fitted for the HRV and cortisol outcome variables. There were three conditions that were compared within each study, Study 1 (*control, definitely, maybe*), Study 2 (*control, high-stress, low-stress*) and Study 3 (*control, high-chance, low-chance*). Even though there were not significant changes in sleep variables between conditions, the current recommendations state that sleep measures should be included as covariates (Stalder et al., 2016). Therefore, objective measures of total sleep time and sleep efficiency were also included in all models. For each heart variability outcome variable, two alternative versions of the full model were fitted. The first version included a random effect of participant and participant × condition interaction, fixed effects of condition, sleep stage, total sleep time, condition × sleep stage, condition × total sleep time, sleep stage × total sleep time, and condition × sleep stage × total sleep time. In the second version, total sleep time was replaced with sleep efficiency. We reduced both versions of the full model using backward elimination at most to one that only included a random effect of participant and fixed effects of condition and sleep stage. We then selected the reduced model with a smaller Akaike Information Criterion as the final model for estimation and inferences.

A similar approach was conducted for the cortisol outcome variables, however the first version of the model included a random effect of participant, fixed effects of condition, total sleep time, condition × total sleep time. In the second version, total sleep time was replaced with sleep efficiency. We then reduced the model using backward elimination at most to one that only included a random effect of participant and a fixed effect of condition. If a main or interaction effect was statistically significant, post-hoc analyses were conducted using Holm's step-down correction for multiple comparisons.

In Study 2, one participant was removed from all analyses due to non-compliance with the study procedures, leaving a final sample of n = 23. Further, polysomnographic sleep recordings were verified to determine whether participants were awake prior to the on-call protocol. If participants were awake, data pertaining to the CAR were removed from the analyses for that particular morning. In Study 1, two participants were awake on one morning (n = 1 '*maybe* condition', n = 1 '*definitely'* condition). In Study 2, one participant was awake on two mornings (n = 1 '*high-stress* condition', n = 1 '*low-stress* condition'), and two participants were awake on one morning (n = 2 '*low-stress* condition'). In Study 3, one participant was awake on two mornings (n = 1 '*high-chance*', n = 1 '*low-chance*' condition) and two participants were awake on one morning (n = 2 '*low-chance*' condition).

To account for the high inter-assay co-efficient of variation for Study 3, additional analyses were performed. Given that each participant's data was analysed using a single assay we were able to calculate a fold-change (i.e., each participants *control* condition was compared to the *high- and low- chance* conditions). This approach eliminated the issue of the high inter-assay coefficient of variation. However, there were no differences in outcomes between the fold-change approach, and the aforementioned statistical approach. Thus, for consistency of reporting, Study 3 data are reported in line with Study 1 and 2 data. Data are reported as mean ± standard error of the mean and statistical significance was set at $p < 0.05$ for all studies.

3. Results

Sleep

The sleep data has previously been reported, Study 1 (Sprajcer et al., 2018c), Study 2 (Sprajcer et al., 2018a), Study 3 (Sprajcer et al., 2018b). In all three studies, 25 variables related to sleep quantity and quality were analysed. Overall, in all three studies there was very little difference between conditions in terms of sleep outcomes (4 of the variables tested showed differences between conditions). On occasions where there were statistically significant differences in sleep outcomes between conditions the difference was not clinically meaningful. For example, in Study 2% of NREM sleep was higher in the *high-stress* condition (76.5 ± 5.3%) compared to the *low-stress* condition (74.4 ± 4.8%). As the differences in sleep between conditions were small (1% more NREM sleep in the high stress condition compared with the low stress condition) (Sprajcer et al., 2018a), the differences may be explained by normal night to night variation in sleep outcomes (Bei et al., 2016), rather than differences between conditions. Further, these studies (Sprajcer et al., 2018a, b; Sprajcer et al., 2018c), also assessed sleep micro-architecture using quantitative EEG analysis (also known as power spectral analysis) to assess more nuanced changes in sleep (Vakulin et al., 2016). There were no significant differences between conditions in all three studies all outcome measures related to quantitative EEG analysis.

3.1. Heart rate variability

All HRV data are shown in Table 3. Full linear mixed-model parameter estimates are shown in Table 5. Across all three studies, sleep stage was a significant factor ($p < 0.001$) for all HRV outcome variables. The main outcomes relevant to differences between conditions for each study are summarised below.

Study 1. There was a main effect of condition for SDNN and RMSSD ($p \leq 0.027$). SDNN ($p = 0.024$) and RMSSD ($p = 0.036$) were lower in the *definitely* condition compared to the *control* condition, but not the *maybe* condition. There were no differences for SDNN and RMSSD between the *definitely* and *maybe* conditions. There were no main effects of condition for mean NN, LF, and HF.

Study 2. There were no main effects of condition for SDNN, RMSSD, LF, and HF. There was a significant main effect of condition for mean NN ($p = 0.037$). However, there were no significant differences between conditions (*control*, *high-stress, low-stress*).

Study 3. There were no main effects of condition for SDNN, RMSSD, LF, and HF. There was a significant main effect of condition for mean NN ($p = 0.020$). However, there were no significant differences between conditions (*control*, *high-chance, low-chance*).

3.2. Cortisol

All cortisol data are shown in Table 4. Full linear mixed-model parameter estimates are shown in Table 6. The main outcomes are summarised below.

Table 3
Heart rate variability outcome variables by sleep stage, condition and study.

Measure	Stage	Study 1 (n = 24)			Study 2 (n = 23)			Study 3 (n = 24)		
		Control	Definitely	Maybe	Control	High-stress	Low-stress	Control	High-chance	Low-chance
mean NN (ms)	S2	1147.1 ± 34.1	1153.6 ± 32.1	1161.9 ± 31.0	1042.0 ± 26.9	1021.9 ± 25.3	1023.7 ± 25.3	1117.5 ± 33.3	1112.2 ± 39.8	1109.3 ± 39.3
	S3	1124.5 ± 34.1	1138.1 ± 33.1	1145.1 ± 31.5	1022.6 ± 25.7	1009.2 ± 24.5	1010.7 ± 25.8	1109.0 ± 31.5	1088.7 ± 30.0	1083.0 ± 30.3
	REM	1099.1 ± 36.9	1099.9 ± 33.8	1100.6 ± 33.2	968.6 ± 27.0	967.1 ± 24.6	969.2 ± 24.6	1064.3 ± 37.6	1050.3 ± 35.9	1064.1 ± 36.1
SDNN (ms)	S2	65.8 ± 5.2	60.7 ± 4.2	64.9 ± 5.3	65.6 ± 4.2	59.7 ± 3.5	61.6 ± 4.0	68.2 ± 5.2	69.8 ± 8.9	68.6 ± 8.8
	S3	52.7 ± 5.3	48.8 ± 4.5	48.0 ± 3.5	67.5 ± 11.4	54.5 ± 3.7	55.1 ± 4.2	59.2 ± 5.2	57.3 ± 5.2	54.2 ± 4.2
	REM	91.9 ± 7.2	80.5 ± 6.3	83.7 ± 6.1	73.3 ± 3.4	73.0 ± 4.1	74.5 ± 5.1	92.5 ± 7.8	90.1 ± 9.9	91.2 ± 9.7
RMSSD (ms)	S2	69.8 ± 6.2	62.8 ± 5.2	69.5 ± 7.7	60.5 ± 5.6	54.6 ± 5.1	57.1 ± 6.2	69.5 ± 7.7	71.3 ± 13.4	70.4 ± 13.5
	S3	58.5 ± 6.6	53.7 ± 4.9	56.7 ± 5.5	74.4 ± 17.7	54.8 ± 5.0	54.3 ± 5.9	70.5 ± 8.4	66.4 ± 8.4	62.0 ± 6.5
	REM	70.4 ± 8.2	64.3 ± 7.3	67.3 ± 7.7	53.5 ± 5.6	54.3 ± 5.5	54.3 ± 6.5	74.6 ± 9.9	71.5 ± 11.4	72.4 ± 11.6
LF (log ms^2)	S2	6.9 ± 0.2	6.6 ± 0.2	6.8 ± 0.2	6.9 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	6.8 ± 0.1	6.7 ± 0.1
	S3	6.2 ± 0.3	6.1 ± 0.2	6.1 ± 0.2	6.7 ± 0.2	6.5 ± 0.1	6.5 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.1 ± 0.1
	REM	7.3 ± 0.2	7.2 ± 0.2	7.3 ± 0.2	7.1 ± 0.1	7.0 ± 0.2	7.0 ± 0.2	7.5 ± 0.2	7.3 ± 0.2	7.4 ± 0.2
HF (log ms^2)	S2	6.6 ± 0.2	6.5 ± 0.1	6.6 ± 0.2	6.5 ± 0.2	6.4 ± 0.2	6.3 ± 0.2	6.8 ± 0.2	6.9 ± 0.2	6.8 ± 0.2
	S3	6.2 ± 0.2	6.2 ± 0.1	6.2 ± 0.2	6.5 ± 0.3	6.4 ± 0.2	6.4 ± 0.2	6.8 ± 0.2	6.7 ± 0.2	6.6 ± 0.2
	REM	6.4 ± 0.2	6.3 ± 0.2	6.5 ± 0.2	6.1 ± 0.2	6.1 ± 0.2	6.1 ± 0.2	6.6 ± 0.2	6.6 ± 0.3	6.6 ± 0.3

Data are reported in mean ± standard error of the mean. mean NN = average time interval between consecutive heartbeats; SDNN = standard deviation of the time interval between consecutive heartbeats; RMSSD = the root mean square of consecutive differences between heartbeats; LF = logarithmic function of low frequency band oscillations; HF = logarithmic function of high frequency band oscillations; S2 = Stage 2 Sleep; S3 = Stage 3 Sleep; REM = Rapid eye movement Sleep.

Study 1. There was a main effect of condition for diurnal cortisol AUC_G ($p = 0.002$). Diurnal AUC_G was also lower in the *definitely* ($p = 0.002$) and *maybe* conditions ($p = 0.002$), when compared to the *control* condition. There were no main effects of condition for CAR AUC_I, post-awakening AUC_G, awakening cortisol concentration, CAR peak or CAR reactivity.

Study 2. There was a main effect of condition for CAR AUC_I ($p = 0.001$) and CAR reactivity ($p = 0.024$. CAR AUC_I was lower in the *high-stress* condition compared with the *control* ($p = 0.015$) and *low-stress* conditions ($p = 0.004$). For CAR AUC_I there was no difference between the *control* and *low-stress* condition. CAR reactivity was higher in the *low-stress* condition compared to the *high-stress* condition ($p = 0.020$), but not when compared to control. For CAR reactivity, there was no difference between the *control* condition and *high-stress* condition.

Study 3. There were no main effects of condition for any cortisol outcome variables.

4. Discussion

This is the first study to investigate how different aspects of being on-call (likelihood of receiving a call, task stress, and chance of missing a call) affect physiological stress outcomes. Three separate but related studies utilised an on-call laboratory simulation to investigate how different characteristics of on-call work impact overnight HRV during sleep and next-day salivary cortisol concentrations. Our hypothesis that increased physiological stress would result when participants were told they would definitely receive a call, perform a high-stress task upon waking or have a high-chance of missing a call was not supported by the data.

To the authors' knowledge, the current study is the first to assess the impact of on-call periods on overnight HRV during sleep. In response to the on-call conditions imposed for all three studies, overnight HRV during sleep was essentially unchanged. The only HRV variable that was significantly different between conditions was in Study 1 where SDNN and RMSSD were lower, across all sleep stages, in the *definitely* condition when compared to *control*. This suggests that when participants knew that they were definitely going to be woken by a call, there was predominant sympathetic modulation and a withdrawal in autonomic nervous system activity during sleep, which indicates an increase in physiological stress (Tobaldini et al., 2013b). In this study, participants also reported feeling more anxious before bed when they were definitely going to be called (Sprajcer et al., 2018c), which may have contributed lower RMSSD during sleep, because anxiety and worry are often associated with the reduction in the parameters related to respiratory sinus arrhythmia (Fisher and Newman, 2013; Thayer et al., 1996). The chronic impact of reduced SDNN and RMSSD during sleep is unknown and requires further investigation, but lower SDNN and RMSSD during wake periods are associated with increased risk for adverse events, including cardiovascular disease and all-cause mortality (Carnethon et al., 2006; Drawz et al., 2013; Pop-Busui et al., 2010; Rautaharju et al., 2006).

As discussed, with the exception of Study 1, findings from the present on-call investigation generally show that HRV during sleep is unaffected during simulated on-call conditions. However, in the on-call context there is an absence of related literature, to which these results can be compared. In the absence of similar studies, differences between study conditions will be discussed in reference to how circumstances that resemble aspects of on-call work, for example anticipating experimental stress (Hall et al., 2004) and daily worry (Brosschot et al., 2007) impact HRV during sleep. A previous study by Hall et al. (2004) found that when compared to anticipating a reading task, anticipating an oral presentation the next morning was associated with decreased levels of parasympathetic modulation (indicating increased physiological stress) throughout the preceding sleeping period. Another study also found that daily worry was associated with increased physiological stress during the sleep (Brosschot et al., 2007). The experimental stress conditions of Hall et al. (2004) were similar to Study 2 of the current investigation, and experiences of pre-bed worry of Brosschot et al. (2007) were simulated in the on-call conditions employed across all studies (Study 1, 2 and 3). In contrast to this prior research, no meaningful changes to HRV during sleep were seen in the present studies. However, it is important to note that these previous findings were not in an on-call context. The lack of change in HRV measures suggest that: 1) there is no anticipatory effect of being on-call, 2) experimental levels of pre-bed stress and/or worry in participants were not sufficient to elicit a physiological response, 3) the methods we employed were not sensitive enough to detect changes between conditions and/or 4) the combination of extraneous factors purposely absent in the laboratory (e.g., noise, light, previous sleep opportunity, family and social commitments) influence stress reported while on-call. Future research is needed to assess HRV during sleep preceding real-world on-call work.

Table 4
Cortisol outcome variables by condition for each study.

Measure	Study 1 (n = 24)			Study 2 (n = 23)			Study 3 (n = 24)		
	Control	Definitely	Maybe	Control	High-stress	Low-stress	Control	High-chance	Low-chance
CAR AUC_{I} $_{0700\text{-}0800h}$ ($nmol{\cdot}h{\cdot}L^{-1}$)	536.0 ± 87.2	644.7 ± 85.8	562.9 ± 88.5	332.4 ± 55.1	199.8 ± 55.1	358.8 ± 55.1	300.0 ± 50.8	304.6 ± 45.3	369.9 ± 48.8
Post-awakening AUC_{G} $_{0700\text{-}0800h}$ ($nmol{\cdot}h{\cdot}L^{-1}$)	1172.2 ± 88.6	1174.3 ± 87.5	1135.3 ± 89.6	678.5 ± 47.9	621.0 ± 47.9	740.6 ± 47.9	709.7 ± 45.4	746.0 ± 40.8	791.5 ± 43.0
Awakening cortisol ($nmol{\cdot}L^{-1}$)	10.7 ± 1.0	8.8 ± 1.0	9.7 ± 1.0	5.8 ± 0.7	7.0 ± 0.7	6.6 ± 0.7	6.0 ± 0.6	6.6 ± 0.5	6.9 ± 0.6
CAR Peak ($nmol{\cdot}L^{-1}$)	28.2 ± 2.3	27.6 ± 2.3	25.4 ± 2.3	15.9 ± 1.2	15.6 ± 1.2	17.9 ± 1.2	15.7 ± 0.9	15.9 ± 0.9	17.5 ± 0.9
CAR Reactivity ($nmol{\cdot}L^{-1}$)	18.6 ± 2.2	18.8 ± 2.2	15.7 ± 2.2	10.5 ± 1.2	9.0 ± 1.2	12.8 ± 1.3	9.3 ± 1.1	9.3 ± 1.1	11.5 ± 1.1
Diurnal cortisol AUC_{G} $_{0700\text{-}1700h}$ ($nmol{\cdot}h{\cdot}L^{-1}$)	5130.6 ± 378.0	4201.3 ± 371.3	4211.2 ± 378.0	2483.0 ± 158.5	2699.7 ± 158.5	2586.9 ± 158.6	2871.1 ± 148.5	2966.5 ± 148.0	2923.9 ± 148.3

Data are reported in mean ± standard error of the mean. AUC_{G} = area under the curve with respect to ground; AUC_{I} = area under the curve with respect to increase; CAR = cortisol awakening response.

Across all three studies, being on-call overnight had some impact on next-day salivary cortisol concentrations. Cortisol outcome variables that reflect the cortisol awakening response (CAR AUC_{I}, post-awakening AUC_{G}, awakening cortisol concentration, CAR peak and CAR reactivity) were unaffected by the on-call conditions imposed across all three studies, with the exception of CAR AUC_{I} and CAR reactivity in Study 2. In this study, CAR AUC_{I} was blunted in the *high-stress* condition, compared to *control* and *low-stress* conditions and, CAR reactivity was higher in the *low-stress* condition, compared with the *high-stress* condition. Previous research has noted that a blunted CAR followed night call outs in fire and emergency service personnel (Hall et al., 2019), and has been associated with depression (Huber et al., 2006), and burnout (Pruessner et al., 1999). In the current study, it is possible that the anticipated stress associated with the *high-stress* condition contributed to this response, however it should also be noted that the CAR AUC_{I} and reactivity measures were within normal ranges for healthy adults (Huber et al., 2006; Pruessner et al., 1999; Westermann et al., 2004). It was somewhat surprising that the anticipation of different aspects of on-call work was associated with few significant changes in CAR responses across studies, especially given previous work has shown that low predictability, novelty and lack of control are associated with higher CAR the following day (Adam et al., 2006; Breier et al., 1987; Kirschbaum and Hellhammer, 1994). However, the lack of anticipatory stress in Studies 1 and 3 is consistent with a recent study investigating cortisol responses during real-life on-call work (Hall et al., 2019). This study showed that post-awakening AUC_{G} and CAR peak were only impacted the morning following a night when a call was actually received by the on-call worker, compared to being on-call without receiving a call, or being off-call (Hall et al., 2019). Furthermore, there were no significant differences in CAR AUC_{I} or CAR reactivity, between on-call and off-call conditions (Hall et al., 2019).

In previous work from our group, participants responding to the same overnight alarm to that used in the current study at 0400 h exhibited higher cortisol CAR peak, CAR reactivity and CAR AUC_{I}, compared to the same conditions without an alarm (Hall et al., 2016). In the current study, during the on-call conditions (except for in the *low-chance* condition of Study 3 where no alarm was sounded) the alarm was sounded at 0700 h. Therefore, the cortisol response to an alarm may be dependent on when the alarm is sounded. Further research is needed to determine whether alarm timing influences CAR responses, especially given that on-call workers can be woken at any time during the night. It is important to note that even though the participants in the current study were told they could be woken at any time during the night, they were always given an 8 -h sleep opportunity. In all studies, it was anticipated that there would be significant sleep decrements on the on-call nights within this 8 -h sleep opportunity. This was a major factor underlying our hypothesis, as impaired sleep can increase physiological stress (Steiger, 2002). Therefore, adequate sleep opportunity may also be a protective factor against the impacts of anticipation of a call on physiological stress. Future studies should compare how on-call conditions with and without adequate sleep impact participants' physiological stress response.

Diurnal cortisol AUC_{G}) was also assessed in the current study. In Study 1, diurnal cortisol AUC_{G} were lower in the on-call conditions (*definitely* and *maybe*) when compared to the *control* condition. There were no differences in diurnal cortisol outcomes in Study 2 or Study 3. This suggests that the different aspects of being on-call may affect the functioning of the hypothalamo-pituitary adrenal axis in different ways and, in light of the lack of differences observed by Hall et al. (2019), may actually mask the effect of each other. This is an important finding, as it is not currently known how two stressors that elicit different stress responses (blunting and heightening of the cortisol profile) interact to affect long-term health. However, it is important to note that the magnitude of these reported changes are small and all cortisol data are still within normal adult reference ranges (Westermann et al., 2004).

Table 5
Linear mixed-effects model parameter estimates for heart rate variability outcome variables.

	Fixed Effects	mean NN	SDNN	RMSSD	LF	HF
Study 1 (n = 24)	Condition	F(2, 38) = 0.46	**F(2, 164) = 3.66***	**F(2, 163) = 3.94***	F(2, 163) = 0.91	F(2, 164) = 1.79
	Sleep Stage	**F(2, 122) = 5.19****	**F(2, 163) = 105.5*****	**F(2, 163) = 12.46*****	**F(2, 163) = 65.78*****	**F(2, 163) = 8.76*****
	EFF / TST	F(1, 41) = 0.06	x	x	x	x
	Sleep Stage x Condition	x	x	x	x	x
	Stage × EFF / TST	**F(2, 122) = 5.29****	x	x	x	x
Study 2 (n = 23)	Condition	**F(2, 168) = 3.35***	F(2, 179) = 1.47	F(2, 179) = 0.80	F(2, 180) = 1.04	F(2, 179) = 0.68
	Sleep Stage	**F(2, 167) = 61.28*****	**F(2, 179) = 60.10*****	**F(2, 179) = 3.33***	**F(2, 179) = 31.61*****	**F(2, 179) = 14.72*****
	EFF / TST	F(1, 172) = 0.01	x	x	x	x
	Sleep Stage x Condition	x	x	x	x	x
	Stage × EFF / TST	**F(2, 168) = 3.21***	x	x	x	x
Study 3 (n = 24)	Condition	**F(2, 182) = 6.04***	F(2, 184) = 0.63	F(2, 184) = 1.25	F(2, 184) = 0.58	F(2, 184) = 0.85
	Sleep Stage	**F(2, 181) = 10.14****	**F(2, 184) = 89.03*****	F(2, 184) = 2.88	**F(2, 184) = 89.04*****	**F(2, 184) = 4.14***
	EFF / TST	F(1, 197) = 1.16	x	x	x	x
	Sleep Stage x Condition	x	x	x	x	x
	Stage × EFF / TST	**F(2, 182) = 6.14***	x	x	x	x

EFF = sleep efficiency; TST = total sleep time.
*** P < 0.001.
** P < 0.01.
* P < 0.05.

This study has some limitations to consider when interpreting the results. The within-subject laboratory based design was employed to isolate the impact of various aspects of on-call on physiological stress. Further, it did appear that participants took the on-call instructions seriously. For example, in Study 2 participants' heart rate increased in anticipation and in response to the speech task (Sprajcer et al., 2018a) and some participants actually woke overnight thinking they heard the alarm during the *high chance* condition in Study 3 (Sprajcer et al., 2018b). However, it is unlikely that on-call conditions in a laboratory environment elicit the same level of physiological stress as real-world on-call conditions, which may, in part, explain the relatively small changes we observed in both overnight HRV and salivary cortisol outcomes. Second, the control night was always first in the protocol and the on-call nights were counterbalanced to reduce the likelihood of the previous nights' conditions impacting subsequent sleep. However, it is possible that the conditions of the previous day did impact overnight HRV and next-day cortisol outcomes in some way. Further, we cannot rule out the possibility that 'last day effects' did not impact stress levels on the final day of the study. Third, additional measurements of respiration rate may have provided more precise HRV during sleep in this sample. Finally, this was an all-male, young-adult sample. Given the known sex differences in both HRV measures during sleep (Elsenbruch et al., 1999) and the cortisol responses to psychological stress (Kirschbaum et al., 1992), future studies should include female participants. In addition, the use of young adults, with no experience of on-call work, may limit the degree to which their data can be generalised to older and/or experienced on-call workers.

Overall, there seemed to be little difference in both HRV outcomes and cortisol variables between conditions in all three studies. It is possible that the on-call environment in the current study was not sufficiently stimulating to elicit changes to overnight HRV and next-day cortisol responses, or there may be no effect of simulated on-call conditions on these measures. Further research is needed where participants engage in real on-call work tasks (e.g., on-call doctors simulating operating procedure) in a laboratory environment to truly isolate and investigate the factors that influence the physiological stress responses to on-call work.

Table 6
Linear mixed-effects model parameter estimates for cortisol outcome variables.

	Fixed Effects	CAR $AUC_{I\ 0700\text{-}0800h}$	Post-awakening $AUC_{G\ 0700\text{-}0800h}$	Awakening cortisol concentration	CAR peak	CAR reactivity	Diurnal cortisol $AUC_{G\ 0700\text{-}1700}$
Study 1 (n = 24)	Condition	F(2, 44) = 0.86	F(2, 43) = 0.17	F(2, 44) = 2.08	F(2, 45) = 0.95	F(2, 44) = 1.28	F(2, 42) = 7.40**
	EFF / TST	x	x	x	x	x	x
	Condition × EFF/TST	x	x	x	x	x	x
Study 2 (n = 23)	Condition	F(2, 46) = 5.22**	F(2, 45) = 2.67	F(2, 44) = 2.46	F(2, 46) = 2.15	F(2, 42) = 4.08*	F(2, 46) = 1.28
	EFF / TST	x	x	x	x	x	x
	Condition × EFF/TST	x	x	x	x	x	x
Study 3 (n = 24)	Condition	F(2, 39) = 0.71	F(2, 41) = 1.04	F(2, 38) = 0.96	F(2, 43) = 1.58	F(2, 37) = 1.67	F(2, 36) = 0.30
	EFF / TST	F(1, 44) = 4.34*	F(1, 48) = 14.62***	x	F(1, 50) = 15.73***	F(1, 45) = 5.77*	x
	Condition × EFF/TST	x	x	x	x	x	x

EFF = sleep efficiency; TST = total sleep time.
*** P < 0.001.
** P < 0.01.
* P < 0.05.

Declaration of Competing Interest

None.

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