Salivary alpha amylase in on-call from home fire and emergency service personnel

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Abstract

The effect of working on-call from home on the sympatho-adrenal medullary system activity is currently unknown. This study had two aims, Aim 1: examine salivary alpha amylase awakening response (AAR) and diurnal salivary alpha amylase (sAA) profile in fire and emergency service workers who operate on-call from home following a night on-call with a call (NIGHT-CALL), a night on-call without a call (NO-CALL) and an off-call night (OFF-CALL), and Aim 2: explore whether there was an anticipatory effect of working on-call from home (ON) compared to when there was an off-call (OFF) on the diurnal sAA profile. Participants wore activity monitors, completed sleep and work diaries and collected seven saliva samples a day for one week. AAR area under the curve with respect to ground (AUC_G), AAR area under the curve with respect to increase (AUC_I), AAR reactivity, diurnal sAA slope, diurnal sAA AUC_G and mean 12-h sAA concentrations were calculated. Separate generalised estimating equation models were constructed for each variable of interest for each aim. For Aim 1, there were no differences between NIGHT-CALL or NO-CALL and OFF-CALL for any response variable. For Aim 2, there was no difference between any response variable of interest when ON the following night compared to when OFF the following night ($n = 14$). These findings suggest that there is no effect of working on-call from home on sAA, but should be interpreted with caution, as overnight data were not collected. Future research, using overnight heart rate monitoring, could help confirm these findings.

Key Words

- salivary alpha amylase awakening response
- diurnal salivary alpha amylase
- sAA
- salivary alpha amylase
- home call
- distal on-call
- irregular work schedules

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Introduction

On-call is a form of irregular work scheduling where personnel are available outside 'regular' work hours (1). Workers operate on-call around the world, with approximately 25% of the workforce in Australia (2) and 20% in the European Union (3) regularly operating with on-call as part of their normal work scheduling. There are two main forms of on-call work: on-call on-site, where workers remain at work while on-call and are usually provided a place to sleep, and on-call from home, where workers are able to leave the workplace and are called, if required. To date, research has typically focussed on on-call on-site work (4), with considerably less research investigating the effects of working on-call from home.

One sector where on-call from home work is particularly important is the fire and emergency services, with over one million personnel operating on-call from

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home (5, 6, 7, 8, 9, 10, 11). Several studies have shown that being on-call from home results in higher subjective stress levels than when not on-call (1, 12, 13, 14, 15, 16). Despite this, few studies have investigated the physiological stress response to operating on-call from home (1, 17, SJ Hall, AI Turner, SA Ferguson, SJ Robertson & B Aisbett, unpublished observations). These studies have focussed on the hypothalamic–pituitary–adrenal axis; no study to date has investigated the effect of working on-call from home on the activity of the sympatho-adrenal medullary (SAM) system.

Studies investigating the effect of working on-call on-site on the SAM system have typically shown that there is a heightened activation when on-call on-site compared to when not on-call (18, 19, 20). For example, the night heart rate has been shown to be approximately 3±2 beats/min higher ($P<0.05$) on nights when ships' engineers were on-call at work compared to nights when sleeping at work but not on-call (20). Samel and coworkers (19) also observed that the 24-h mean urine excretion rates of adrenaline and noradrenaline of emergency helicopter pilots were up to 153±49% and 158±49% higher ($P\leq0.01$), respectively, when on-call at work compared to when off-duty at home. Similarly, research conducted by Ernst and coworkers (18) observed that the concentration of noradrenaline in doctors' urine was approximately 10 µg higher ($P<0.01$) every 24 h when on-call at work compared to when not on-call. Although the collection of blood and urine is possible in the on-call on-site setting, it may not be as practical or convenient when collecting data from personnel working on-call from home. The stability of urine and blood samples is dependent on the temperature during the collection period, which is difficult to control across a number of locations (21) and it is recommended that samples should be frozen immediately following collection (22), which may not be possible for fire and emergency service personnel operating on-call at home as they often have another job during the day (9), and may not have immediate access to a freezer.

Pilot work, investigating the feasibility of heart rate monitoring for this cohort, identified that commercially available heart rate monitors often beep when conductivity is poor (SJ Hall & B Aisbett, unpublished observations). This is often the case when worn for extended periods. Therefore, they are not appropriate for use where disrupted sleep is a variable of interest, as is the case in the current study. Other monitors such as Actiheart have the potential to be used in on-call from home populations, as they can be worn for longer periods (23), although they require correct fitting and anatomical knowledge (24). This is not feasible in remote rural locations, which is where the majority of volunteer on-call from home firefighters reside (25). Consequently, another physiological marker, salivary alpha amylase (sAA), which is stable up to 37°C for up to three weeks (26), is more feasible for these settings.

sAA has been shown to follow a diurnal pattern (27). Concentrations drop sharply upon awakening, which is followed by a gradual increase across the day (28). Thoma and coworkers (29) have shown a rise in awakening sAA concentrations, instead of the typical drop, in post-traumatic stress disorder patients and Rohleder and coworkers (30) demonstrated that chronic stress may be associated with a flattening of the diurnal sAA profile in a field setting in familial caregivers of patients with brain cancer. Repeated and long-term activation of the SAM system may result in an increased risk of adverse health outcomes, such as hypertension, coronary heart disease and anxiety (31). Therefore, analyses of the salivary alpha amylase awakening response (AAR) and diurnal sAA pattern may help to identify whether agencies should consider interventions to mitigate potential stress-related health issues resulting from operating on-call from home.

This study had two main aims. Aim 1: to establish the effect of working on-call from home on the AAR and diurnal sAA profile of fire and emergency service workers the day following a night (i) on-call from home with a night call (NIGHT-CALL), (ii) on-call from home without a night call (NO-CALL) and (iii) off-call (OFF-CALL). We hypothesised that the AAR and diurnal sAA profiles would both be altered following NIGHT-CALL and NO-CALL, compared to OFF-CALL, reflected by a larger AAR AUC_G, a less negative AAR AUC_I, higher AAR maximum response, smaller AAR reactivity, flatter diurnal sAA slope, higher 12-h mean sAA and larger diurnal sAA AUC_G when on-call. Aim 2: to determine whether there is an anticipatory effect on diurnal sAA profiles, 'evening' sAA levels (12-h post-awakening) and AAR maximum response when operating on-call from home the following night (ON) compared to when off-call the following night (OFF). Please note that 'following night' refers to the night immediately following the day where samples were taken. We hypothesised that evening sAA levels and AAR maximum response would be higher and consequently, the 12-h mean sAA concentrations and sAA diurnal AUC_G would be higher, but that no differences would be observed in diurnal sAA slope the day before an ON night compared to the day before an OFF night.

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Materials and methods

Participants and recruitment

Prior to recruitment, the study protocol was approved by the Deakin University Human Ethics Committee (project ID 2014-278). Recruitment fliers were sent to Australian fire and state emergency service (SES) agencies for distribution to personnel (salaried and volunteer), researchers visited fire brigades and SES units and advertisements were placed in agency-based newsletters and magazines and on social media sites. Interested personnel contacted researchers directly for further information about the study.

The inclusion criteria specified that participants should be male on-call fire or emergency service workers aged 18–75 years. Seventy-eight fire and emergency service personnel provided written informed consent. Participants were excluded if they had an injury or condition that prevented them from performing their normal on-call duties, had a diagnosed sleep condition that was not currently being treated, were suffering from a contagious illness, were taking steroid medication or worked on-call from home in another profession. Four potential participants were excluded based on these criteria. Ten personnel were unavailable during the study period (e.g. sick, injured or on holidays), so they were not sent study kits.

Study kits were sent to sixty-four fire and emergency service personnel. Of these, two participants revoked consent and 14 did not return their kits. Of the remaining 48, the data sets of two participants were excluded because of inconsistencies between sleep and work diaries and/or irregularities with their stress data (e.g. samples not stored correctly during data collection). Finally, if participants did not have data pertaining to two or more on-call conditions, they were excluded. Thus, the final data set contained 26 fire and emergency service personnel for Aim 1 and 14 for Aim 2. There were no significant differences in demographic characteristics between fire and emergency service workers that were included or excluded from the analyses (data not shown), and demographics were similar to previous studies in this population (32, 33). Consequently, it is unlikely that any bias was introduced due to this inclusion method.

Experimental protocol

Study kits, comprising instructions on how to complete the study, an Actical activity monitor (MiniMitter/Respironics, Bend, OR, USA), a sleep diary, a work diary, salivettes (Sarstedt, Nurnbrect, Germany) and reply paid return-addressed envelopes were mailed to participants. Participants were instructed to wear the activity monitor, complete the daily sleep and work diaries and collect saliva samples for one week. Participants also wore the activity monitor and completed the daily sleep and work diaries for a second week, which will be published elsewhere with a more detailed analysis of sleep data (SJ Hall, B Aisbett, AI Turner, SJ Robertson & SA Ferguson, unpublished observations). Participants knew in advance whether they were going to be on-call or not, with most either operating on-call 24/7 or having set rosters for their on-call work (for example, one week on-call followed by three weeks off-call).

Participants completed a custom-made sleep diary, adapted from Vincent and coworkers (33, 34) for any sleep or attempted sleep and completed information pertaining to the start and end time of each sleep period or attempted sleep period, the sleeping location, the number of times they woke during the sleep and an estimate of their total sleep time and their subjective sleep quality. A 6-point Likert scale, modified from Vincent and coworkers (33, 34), was used to assess subjective sleep quality, where 1='very good', 2='good', 3='average', 4='poor', 5='very poor' and 6='did not sleep'. Other data collected in the sleep diary comprised self-report height and weight (used to calculate body mass index; BMI), age, years of fire and emergency service experience, smoking status and average daily caffeinated beverage consumption (number of cups). The start and end times of regular work periods, location of work, the start and end time of on-call periods and the time of, and type of any call out were recorded using the daily work diary.

An activity monitor (Actical, MiniMitter/Respironics) was worn by participants on their non-dominant wrist, as an objective, validated, indirect measure of sleep (35). Participants were asked to press the event marker button on the activity monitor each time they collected a saliva sample, so that the exact timing of sampling was recorded and were instructed to remove the activity monitor if it was likely to get wet. The activity monitors were set to record at 1-min epochs. Raw activity scores were downloaded using a specialised interface unit (ActiReader, Respironics, Bend, OR, USA) and were translated into sleep–wake scores by a validated manufacturer propriety algorithm to infer sleep–wake measures (Actical v3.10), with a sensitivity of <40 counts/epoch used to distinguish between sleep and wake states (35).

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Saliva samples were collected using salivettes 0, 30, 60 min, 3, 6, 9 and 12 h after awakening. This is consistent with recommendations for the measurement of the diurnal sAA profile (28). To reduce participant's burden and enhance compliance, a text message reminder system was developed. When participants woke, they entered their awakening time on a unique website; they then received text message reminders for all subsequent samples that day, which reminded them to collect their sample and press the event maker on their activity monitor. Once collected, participants were asked to store the samples in their refrigerator until the completion of the study and then mail the samples to Deakin University in a 'return-addressed' prepaid postage bag.

sAA analysis and calculations

Concentrations of sAA were measured at Deakin University using a kinetic assay kit (Salimetrics, Carlsbad, CA, USA), as per the manufacturer's instructions. Concentrations of alpha amylase are reported as enzyme units per millilitre (U/mL), which is the most commonly used unit of measurement of sAA (36). The intra-assay coefficient of variation was 3.8% at 38.5 U/mL, 2.9% at 130.9 U/mL and 2.9% at 105.7 U/mL. The inter-assay coefficient of variation was 10.1% at 40.7 U/mL, 10.9% at 134.5 U/mL and 6.3% at 112.0 U/mL. Saliva samples were also analysed for cortisol concentrations, which will be published elsewhere (SJ Hall, AI Turner, SA Ferguson, SJ Robertson & B Aisbett, unpublished observations).

Compliance was defined as taking the saliva sample (i.e. pressing the activity monitor marker) within 15 min of the intended sampling time for the AAR measures (0, 30 and 60-min samples) and within 1 h of the intended time for the diurnal sAA measures (3, 6, 9 and 12-h samples), in accordance with the criteria used by Broderick and coworkers (37). Non-compliant samples were removed from the analysis. If the activity monitor was not pressed, the sample remained in the analysis. Compliance rates observed in this study were consistent with those observed by Kudielka and coworkers (38).

The sAA responses were assessed using two approaches: AAR and diurnal sAA. AAR was assessed as AUC_G of the 0, 30 and 60-min samples, using the trapezoidal method (Equation 2 from Pruessner and coworkers (39), with time in hours). The diurnal sAA profile was investigated using the 0-min, 3, 6, 9 and 12-h samples. The number of samples utilised and sampling time points have been deemed appropriate for the assessment of diurnal sAA levels (28). Diurnal profile was assessed by diurnal sAA slope, 12-h mean sAA levels and diurnal sAA AUC_G. Diurnal slope was calculated by fitting a line of best fit to the five aforementioned sAA values, the 12-h mean sAA concentration was calculated as the mean of the five aforementioned samples and diurnal sAA AUC_G was

Table 1 Self-reported participant characteristics.

Characteristic	Mean ± S.D.	Range
Age (years)	37 ± 10	20–56
Height (cm)	181 ± 8	166–195
Weight (kg)	89 ± 18	61–130
Body mass index (kg/m^2)	27 ± 5	21–39
Caffeinated beverage consumption (reported as cups per day)	3 ± 2	0–6
Length of service (years)	18 ± 14	2–35

Note: $n = 26$.

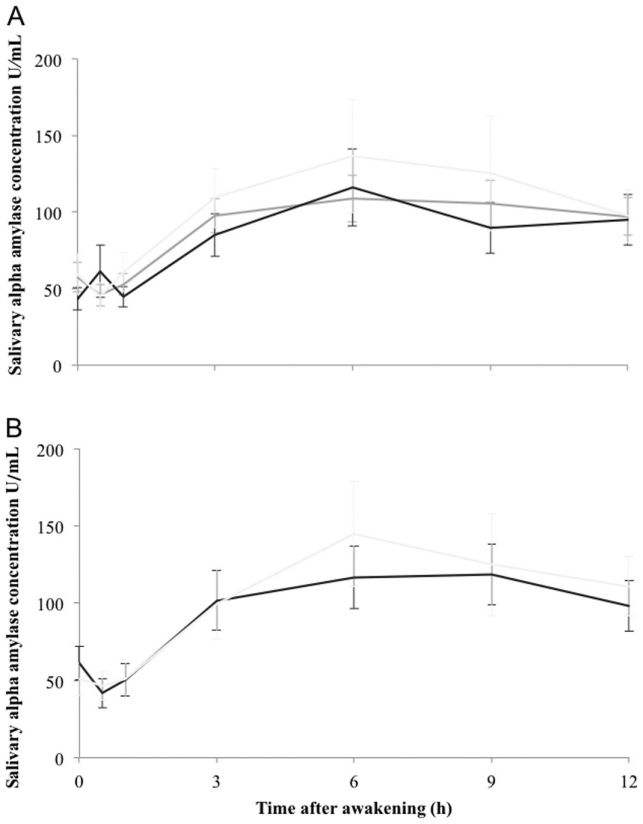

Figure 1
Salivary alpha amylase awakening response and diurnal profile in on-call from home fire and emergency service workers (A) for Aim 1, the day following on-call work ($n = 26$) and (B) for Aim 2, the day prior to on-call work ($n = 14$). Note: data presented as mean ± S.E.M. of individual averages; dark grey – denotes off-call the previous night (OFF-CALL) and denotes off-call the following night (OFF); mid grey – denotes on-call without a call the previous night (NO-CALL); light grey – denotes on-call with a night call the previous night (NIGHT-CALL) and denotes on-call the following night (ON).

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Table 2 Summary of generalised estimating equation models for Aim 1 salivary alpha amylase awakening response (AAR).

	Model 1: AAR AUC_G(Uh/mL)				Model 2: AAR AUC_I(Uh/mL)				Model 3: sAA 30-min sample(U/mL)				Model 4: AAR reactivity(U/mL)			
Parameter	β	S.E.	χ^2	*P*	β	S.E.	χ^2	*P*	β	S.E.	χ^2	*P*	β	S.E.	χ^2	*P*
NIGHT-CALL†	−1.5	10.0	0.0	0.88	5.5	9.4	0.3	0.56	9.5	15.0	0.4	0.53	14.3	16.9	0.7	0.40
NO-CALL†	−16.5	9.6	2.9	0.09	−9.0	6.2	2.1	0.15	−16.5	11.9	1.9	0.17	−8.2	9.6	0.7	0.40
Weekend‡	5.6	5.4	1.1	0.30	8.0	6.1	1.7	0.19	12.1	6.7	3.3	0.07	11.1	9.1	1.5	0.22
Age (years)	1.1	0.5	4.9	0.03*	−0.0	0.4	0.0	0.98	1.2	0.6	4.2	0.04*	−0.1	0.6	0.1	0.81
BMI (kg/m^2)	−0.3	1.2	0.1	0.78	−1.4	1.1	1.6	0.21	−0.6	1.0	0.4	0.53	−2.7	2.0	1.7	0.19
TST (min)	0.0	0.0	0.3	0.60	0.0	0.0	0.0	0.97	−0.03	0.0	0.8	0.36	−0.1	0.0	1.3	0.25
Sleep quality (arbitrary units)	0.0	1.7	0.0	0.98	1.1	2.6	0.2	0.69	−1.1	2.4	0.2	0.65	1.2	4.4	0.1	0.79
Model performance	QIC = 193,441				QIC = 177,835				QIC = 225,791				QIC = 310,365			

β, beta coefficient; χ^2, Walds chi-square; AAR, salivary alpha amylase awakening response; AUC_I, Area under the curve with respect to increase; BMI, Body Mass Index; NIGHT-CALL, on-call with a night call the previous night; NO-CALL, on-call without a night call the previous night; QIC, Quasi-Akaike Information Criterion; sAA, salivary α-amylase; S.E., standard error; TST, total sleep time; sleep quality, subjective sleep quality.
†Denotes compared to off-call (OFF-CALL); ‡denotes compared to weekday; *denotes $P<0.05$; $n=26$.

calculated using the trapezoidal method (Equation 2 from Pruessner and coworkers (39), with time in hours).

Statistical analysis

All statistical analyses were completed using the R computing environment (R Foundation for Statistical Computing, Version 3.1.2, Vienna, Austria) using within-subject generalised estimating equations (GEEs) using the geepack package (Version 1.2–1) and an exchangeable correlation structure for each model. GEEs were deemed appropriate because they are able to manage data with uneven numbers of instances observed across participants and because we expected correlations between repeated measures, which GEEs are able to handle (40). For Aim 1, data were analysed using a separate model for each variable of interest (AAR AUC_G, AAR AUC_I, 30-min sample concentration, AAR reactivity, sAA AUC_G, mean 12-h sAA concentration, diurnal sAA slope, diurnal sAA AUC_G), with an on-call condition considered a three-level fixed factor (ON-CALL, NO-CALL and OFF-CALL), and OFF-CALL used as the reference category for comparison. Age (years), BMI (kg/m^2), and previous night's total sleep time (min), whether it was a weekday or weekend and subjective sleep quality (6-point Likert scale) for the main sleep, were entered as covariates within each model. Data for Aim 2 were analysed using a separate model for each sAA variable of interest (mean 12-h sAA concentration, diurnal sAA slope, diurnal sAA AUC_G, 12-h sAA sample concentration), accounting for previous night's call condition, with following night's on-call condition as the fixed factor (ON or OFF), with OFF as the reference category for comparison. Age, BMI, whether it was a weekday or weekend, and subjective sleep quality were entered as covariates within each model. Statistical significance was set at $P<0.05$ and all data are presented as mean ± standard error (S.E.) of the mean unless otherwise stated.

Results

Participant characteristics are shown in Table 1. The final sample included participants from seven Australian fire and emergency service agencies. Based on self-report data, 17 were non-smokers, seven were ex-smokers, one was an occasional smoker (i.e. not every day) and one was a regular smoker (smokes every day). Twenty-three participants were employed in full-time positions, two worked part-time and one did not work. In total, 1181 saliva samples were collected with a compliance rate of 96.8%.

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Table 3 Summary of generalised estimating equation models for Aim 1 diurnal salivary alpha amylase (sAA) profile.

	Model 5: Mean 12-h sAA(U/mL)				Model 6: Diurnal sAA slope(U/mL/h)				Model 7: Diurnal sAA AUC_G(Uh/mL)			
Parameter	β	S.E.	χ^2	***P***	β	S.E.	χ^2	***P***	β	S.E.	χ^2	***P***
NIGHT-CALL†	−6.1	10.3	0.4	0.55	0.4	1.4	0.1	0.79	−146.9	116.0	1.6	0.21
NO-CALL†	−7.7	10.9	0.5	0.48	−1.3	1.4	0.8	0.37	−94.6	113.5	0.7	0.41
Weekend‡	5.2	6.6	0.6	0.43	0.5	0.7	0.5	0.48	90.2	84.2	1.1	0.28
Age (years)	2.2	1.2	3.6	0.06	0.1	0.1	1.8	0.18	29.7	14.6	4.1	0.04*
BMI (kg/m^2)	2.2	2.3	1.0	0.32	−0.2	0.1	1.8	0.17	31.5	30.4	1.1	0.30
TST (min)	0.0	0.1	0.6	0.44	0.0	0.0	0.6	0.44	0.5	0.7	0.5	0.46
Sleep quality (arbitrary units)	−1.6	4.6	0.1	0.73	−0.2	0.5	0.2	0.65	−23.3	61.7	0.1	0.71
Model performance	QIC = 485,685				QIC = 3867				QIC = 722,171			

β, beta coefficient; χ^2, Walds chi-square; AUC_G, Area under the curve with respect to ground; BMI, Body Mass Index; NIGHT-CALL, on-call with a night call the previous night; NO-CALL, on-call without a night call the previous night; QIC, Quasi-Akaike Information Criterion; sAA, salivary alpha amylase; S.E., standard error; TST, total sleep time; sleep quality, subjective sleep quality.
†Denotes compared to off-call (OFF-CALL); ‡ denotes compared to weekday; * denotes $P<0.05$; $n=26$.

Aim 1: sAA following on-call nights

The analysis for Aim 1 comprised 110 nights ($n=26$): 22 OFF-CALL nights, 68 NO-CALL and 20 NIGHT-CALL nights. Figure 1A shows sAA concentrations over time and Tables 2 and 3 provide the outcomes for each GEE model for Aim 1. Call condition was not a significant predictor for AAR AUC_G (Model 1), AAR AUC_I (Model 2), AAR 30-min sample concentration (Model 3), AAR reactivity (Model 4), mean 12-h sAA (Model 5), diurnal sAA slope (Model 6) or diurnal sAA AUC_G (Model 7; Tables 2 and 3). Age was positively associated with AAR AUC_G, AAR 30-min sample concentration and diurnal sAA AUC_G (Tables 2 and 3). There were no other significant contributors to any of the aforementioned models.

Aim 2: sAA in anticipation of a night on-call

The analysis for Aim 2 included 117 nights ($n=14$): 25 OFF and 92 ON nights. Figure 1B shows sAA concentrations over time (including the 30-min and 60-min samples, which were not included in the Aim 2 analysis) and Table 4 provides the outcomes of each GEE model for Aim 2. Call condition was not a significant predictor for mean 12-h sAA (Model 8), diurnal sAA slope (Model 9) or diurnal sAA AUC_G (Model 10) or the 12-h sAA sample concentration (Model 11; Table 4). Age was positively associated with mean 12-h sAA and diurnal sAA AUC_G (Table 4). BMI was negatively associated with the 12-h sAA sample concentration (Table 4). There were no other significant contributors to any of the Aim 2 models (Table 4).

Discussion

To our knowledge, this is the first study to investigate the effect of working on-call from home on the activity of the SAM system. Contrary to our hypothesis for Aim 1, there was no difference in any sAA measure the day following NIGHT-CALL and NO-CALL compared to OFF-CALL. Contrary to our hypothesis for Aim 2, there was no difference in the 12-h sAA sample concentration or the diurnal sAA slope when ON the following night compared to when OFF the following night. As expected, there were no differences in the mean 12-h sAA concentration or the diurnal sAA AUC_G when ON the following night compared to when OFF the following night.

Based on previous work conducted in on-call on-site occupations, which showed heightened 24-h adrenaline and noradrenaline when on-call (18, 19), it was expected that 12-h mean sAA would be increased following

Table 4 Summary of generalised estimating equation models for Aim 2 diurnal salivary alpha amylase (sAA) profile.

	Model 8:Mean 12-h sAA(U/mL)				Model 9:Diurnal sAA slope(U/mL/h)				Model 10:Diurnal sAA AUC_G(Uh/mL)				Model 11:12-h sAA sample(U/mL)			
Parameter	β	S.E.	χ^2	***P***	β	S.E.	χ^2	***P***	β	S.E.	χ^2	***P***	β	S.E.	χ^2	***P***
ON[†]	−13.0	12.2	1.1	0.29	−3.1	2.1	2.2	0.14	−124.5	155.2	0.6	0.42	−18.9	22.1	0.7	0.39
Weekend[‡]	6.4	7.5	0.7	0.40	−0.3	1.9	0.0	0.86	104.7	90.1	1.4	0.25	−2.0	18.7	0.0	0.92
Age (years)	4.5	1.4	9.8	<0.01*	0.0	0.1	0.1	0.76	62.0	17.9	12.0	<0.01*	0.9	1.2	0.5	0.49
BMI (kg/m^2)	1.7	2.9	0.3	0.56	−0.1	0.2	0.6	0.43	37.6	37.3	1.0	0.31	−7.6	2.2	12.2	<0.01*
Sleep quality (arbitrary units)	−0.0	0.1	0.0	0.98	−0.0	0.0	0.0	0.90	0.4	1.2	0.1	0.71	−0.0	0.1	0.1	0.82
Model performance			QIC = 309,159				QIC = 2438				QIC = 407,462				QIC = 383,401	

β, beta coefficient; χ^2, Walds chi-square; AUC_G, Area under the curve with respect to ground; BMI, Body Mass Index; ON-CALL, on-call the following night; QIC, Quasi-Akaike Information Criterion; sAA, salivary alpha amylase; S.E., standard error; TST, total sleep time; sleep quality, subjective sleep quality.
[†]Denotes compared to off-call (OFF-CALL); [‡]denotes compared to weekday; *denotes $P<0.05$; $n=14$.

NIGHT-CALL and NO-CALL compared to when OFF-CALL. However, this was not observed. The current study only investigated 12-h mean concentrations rather than 24-h concentrations, as it is not possible to collect overnight saliva samples without waking participants, which would have confounded the results. Therefore, it is conceivable that overnight sAA concentrations may have been elevated and that this would have resulted in higher 24-h means had these been investigated. Alternatively, it is possible that there was no difference in mean 12-h sAA concentrations because the SAM system was able to adapt and recover quickly to the stress allowing the body to return to a state of homeostasis prior to next day sample collection. Despite the limitations associated with the collection and storage of urine samples (21, 22) for remote personnel, future research could attempt to collect 24-h urine or morning urine for catecholamine analysis in local on-call from home cohorts to investigate overnight SAM system activity.

Previous research has shown increased sAA in anticipation of academic examinations (41). As a result, it was expected that the 12-h sample would be higher when ON due to the anticipation of a potential overnight emergency response call. However, there was no difference in the 12-h sample concentration prior to ON compared to OFF. This suggests that there may not have been an anticipatory effect of being on-call from home on sAA. Alternatively, it is possible that the 12-h sample is not close enough to bedtime to observe an anticipatory response. Thus, future research could collect samples in the lead up to bedtime to better understand whether there is an anticipatory effect to being on-call from home.

The findings of the current study demonstrated no difference in AAR AUC_G, AAR AUC_I, the 30-min sample concentration or AAR reactivity following NO-CALL or NIGHT-CALL compared to OFF-CALL. These findings should be interpreted with caution. The results could suggest that (a) there is no anticipatory effect to operating on-call from home (NO-CALL vs OFF-CALL), (b) this system returned to homeostasis prior to final morning awakening or (c) there was too much noise associated with these measures. Future research could investigate the acute sAA response to overnight emergency service work to better understand the lack of difference between NIGHT-CALL and OFF-CALL the morning after these on-call conditions. It would also be beneficial for future work to investigate the sAA/cortisol ratio, as this may provide insight into the relationship between the two main physiological stress systems (42).

A limitation of the current study is the inability to control for the varying degree of physical work and

psychological stress experienced by our participants when attending a callout. It is not possible to standardise this in a real-world setting. Furthermore, due to the limited sample size, we were unable to control for the time of year or weather conditions, which may have affected the anticipation to a call. For example, fires are more likely when it is hot and windy (43) and car crashes are more likely under wet conditions (44). Collection of perceived stress measures taken in parallel to the physiological data could have provided further insight and would be a worthwhile inclusion in future studies. However, it should be noted that this would increase participant's burden, and researchers will need to weigh the cost/benefit of this addition. Despite these potential limitations, the real-world setting is considered one of the principal strengths of the current study as replicating on-call from home work in a laboratory also presents difficulties (45). For example, it is unlikely that simulating an on-call from home environment would be able to reproduce the same level of importance and consequence as a real-world setting. Furthermore, sleeping in a laboratory more closely reflects conditions representative of on-call on-site, or proximal on-call work, and not the on-call from home work performed by more than a million emergency service workers across the world.

In light of the limitations associated with the use of heart rate monitors (24; SJ Hall & B Aisbett, unpublished observations) and catecholamine sampling (21, 22) in remote field settings, and the emergence of sAA as a marker of sympathetic activity (46), we decided that sAA would be a suitable measure for investigating the effect of operating on-call from home on activity of the SAM system. When investigating the cortisol awakening response, researchers typically investigate the CAR AUC_G, AUC_I, peak and reactivity. Given the inverse response of α-amylase to awakening, it was expected that AUC_G, AUC_I, trough and reactivity would be suitable to be investigated, particularly as some of these measures have been previously investigated. However, we have identified a potential problem with this approach. We observed a range of responses (in direction, timing and magnitude) in the 0, 30 and 60-min samples. This makes AAR AUC_I, AAR trough and AAR reactivity difficult to analyse. For example, if the trough of AAR is less negative (indicated by a positive β) than the control condition, it could be considered a blunted response. Alternatively, the response may actually be a peak rather than a trough (i.e. sAA has gone up upon awakening), which may indicate a different response entirely. Likewise, AUC_I would normally be expected to be negative due to the decrease in sAA following morning awakening. Thus, a more negative AUC_I could be seen as an augmented response. However, a positive AUC_I could also be seen as atypical. This makes interpreting results from statistical models problematic, particularly given that it is not currently well understood how deviations in either direction may predict negative long-term health outcomes (29).

Since sAA concentrations showed bidirectional movement and varying magnitudes (i.e. noise) in the first 60 min after awakening, AAR may not be as useful as we anticipated. The noise demonstrated in our data is unlikely to be washed out by the addition of more participants. So, although we believe there is still potential to use sAA in future research, we also believe that its role in healthy populations needs to be better understood before it is used in other specialist cohorts. Researchers have begun to unpack potential confounders such as age, gender, BMI, emotion/stress, physical activity and eating and drinking (27). In the current study, age was positively associated with AAR AUC_G and diurnal sAA AUC_G in the models for Aim 1 and mean 12-h sAA, diurnal sAA AUC_G and diurnal AUC_G in the models for Aim 2. This supports the findings of Strahler and coworkers (47), which showed elevated sAA in older adults. BMI was negatively associated with the 12-h sample in Model 11 for Aim 2. To the author's knowledge, no study has investigated the association between BMI and evening sAA before now. Furthermore, a guidelines' paper for collection, analysis and interpretation of results, like the CAR guidelines paper by Stalder and coworkers (48), would be useful before this measure can be more readily applied, as there remains inconsistencies in how researchers collect, store, process and analyse data. For example, Ghiciuc and coworkers (49) removed those that had a flat CAR from the sAA analysis, whereas Nater and coworkers (27) removed samples that were taken more than 10 min before or after the intended sampling time. The impact of these methodological issues on AAR assessment is currently unclear.

Investigating the effect of working on-call from home on activity of the SAM system using remote monitoring seems to pose a number of complex issues. Until more is understood about the sAA and its confounders, it may be worth revisiting the use of Actiheart (or similar) and in more local on-call from home cohorts to better understand the effects of working on-call from home on activity of the SAM system. Likewise, catecholamines may be difficult to monitor (in serum or urine) in remote cohorts; however, if a local cohort were investigated, urine could be picked up soon after sampling to reduce these complications. The drawback of these methods is that the large number of rural on-call from home workers, such as fire and emergency service workers, would be difficult to investigate.

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In summary, we showed that the following day, AAR and diurnal sAA activities were not altered following either on-call condition (NIGHT-CALL or NO-CALL), compared to OFF-CALL. In addition, there was no anticipatory effect of working on-call from home on diurnal sAA measures or the 12-h sample concentration the day prior to a night ON compared to OFF. Investigating the AAR in on-call from home populations in the field seems to be inherently impacted by the variability of sAA in response to other potential confounders. Until more is known about the health impacts and potential confounders associated with sAA and a guidelines' paper has been developed, it may be worth investigating AAR to simulated on-call conditions in a laboratory setting (which has its own set of limitations) to account for some of the context variability and to use heart rate and catecholamine monitoring in local field settings to better understand the effect of working on-call from home on activity of the SAM system.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
Miss Hall contributed to the study design, recruitment, data collection, data analysis, data processing, statistical analysis, interpretation of results and preparation of the manuscript. Associate Professor Aisbett contributed to the study design, recruitment, data processing, interpretation of results and preparation of the manuscript. Professor Ferguson contributed to the study design, recruitment and preparation of the manuscript. Associate Professor Robertson contributed to the study design, statistical analysis and preparation of the manuscript. Dr Turner contributed to the study design, data analysis, data processing, interpretation of results and preparation of the manuscript.

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