Decreased salivary alpha-amylase levels are associated with performance deficits during sleep loss

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Abstract

During sleep deprivation, neurobehavioral functions requiring sustained levels of attention and alertness are significantly impaired. Discrepancies between subjective measures of sleepiness and objective performance during sustained operations have led to interest in physiological monitoring of operator performance. Alertness, vigilance, and arousal are modulated by the wake-promoting actions of the central noradrenergic system. Salivary alpha-amylase (sAA) has been proposed as a sensitive peripheral measure of noradrenergic activity, but limited research has investigated the relationship between sAA and performance. In a laboratory-controlled environment, we investigated the relationship between sAA levels, subjective sleepiness, and performance during two days (50 h) of total sleep deprivation. Beginning at 09:00, twelve healthy participants (5 females) aged 22.5 ± 2.5 years (mean $\pm$ SD) provided saliva samples, recorded ratings of subjective sleepiness, completed a brief 3-min psychomotor vigilance task (PVT-B) and performed a 40-min simulated driving task, at regular 3 h intervals during wakefulness. Ratings of subjective sleepiness exhibited a constant linear increase ($p < 0.001$) during sleep deprivation. In contrast, sAA levels showed a marked diurnal profile, with levels increasing during the day ($p < 0.001$) and steadily declining in the evening and early-morning ($p < 0.001$). PVT-B (mean reaction time and mean slowest 10% reaction time) and simulated driving performance (speed deviation and lane deviation) also exhibited diurnal profiles across the two days of sleep deprivation. Performance peaked in the afternoon ($p < 0.001$) and then steadily worsened as wakefulness continued into the evening and early-morning ($p < 0.001$). Further analysis revealed that higher sAA levels in the hour preceding each performance assessment were associated with better PVT-B and driving performance ($p < 0.001$). These findings suggest that sAA measures may be suitable indicators of performance deficits during sustained wakefulness and highlight the potential for sAA to be considered for physiological monitoring of performance. In operational environments sAA levels, as part of a panel of physiological measures, may be useful for assessing fitness-for-duty prior to safety being compromised or when performance deficits are unknown.

Keywords

Amylase; Saliva; Biomarker; Performance; Sleep deprivation; Sleepiness

1. Introduction

Sleep loss has been extensively associated with impaired vigilance, alertness, and attention (Van Dongen et al., 2003). Performance is mediated by the interaction of the homeostatic pressure for sleep, which increases with time awake and dissipates during sleep, and the diurnal rhythm for alertness that declines during the evening (Achermann and Borbely, 1994). During extended wakefulness or work at night, sleepiness increases and performance is impaired as a result of the increased homeostatic pressure for sleep and the reduced diurnal drive for alertness (Dijk et al., 1992; Van Dongen and Dinges, 2003). The detrimental effects of increased sleepiness on operator performance, as a result of extended work shifts or working during the night, have been documented in transportation (Huffmyer et al., 2016), the military (Lieberman et al., 2005), and medical professions (Barger et al., 2005). In these settings, continual monitoring of sleepiness and performance is important as impaired performance can lead to serious safety compromises with potentially life-threatening consequences. Currently, self-assessment of sleepiness or performance during extended wakefulness is the most common method of monitoring performance and safety in the workplace (Christodoulou, 2012). However, inter-individual differences in the effects of sleep loss on performance (Van Dongen et al., 2004) and reports of discrepancies between subjective assessment of sleepiness and objective performance (Paech et al., 2016; Zhou et al., 2012), have led to the recent interest in non-invasive, objective, physiological monitoring of operator performance.

Physiological measures such as electroencephalography (EEG) recordings (Horne and Baulk, 2004), electrooculography (EOG) metrics (Jackson et al., 2016), and heart rate variability (Li and Chung, 2013), have been identified as effective objective methods of assessing sleepiness and performance during sleep deprivation. However, instrumentational requirements and confounding factors may limit the implementation of these measures in the field. Thus, it may be that a multimodal method with a panel of physiological markers be a more suitable approach for monitoring performance in the field during extended wakefulness. The non-invasive and simple handling of saliva collection has raised interest in the investigation of salivary components as measures of physiological changes (Chiappin et

al., 2007). In the current study, we investigated a salivary marker, alpha-amylase, as a potential measure to be considered for physiological monitoring of performance during sleep deprivation.

Salivary alpha-amylase (sAA) is one of the principal proteins produced by the salivary glands, and has been proposed as a non-invasive peripheral measure of noradrenergic activity (see review by Nater and Rohleder, 2009; van Stegeren et al., 2006). Animal and human studies have revealed that noradrenergic activity is closely linked with alertness and vigilance (Liu et al., 2009; Smith and Nutt, 1996). Additionally, numerous studies have also identified the role of noradrenaline (NA) in mediating sAA secretion and activity (see review by Nater and Rohleder, 2009). For example, isoproterenol (beta-adrenoceptor agonist) treatment of rat parotid acinar cells induced dramatic increases in alpha-amylase secretion (Chen et al., 2014). In humans, propranolol (beta-adrenoceptor antagonist) treatment significantly attenuated increases in sAA levels compared to placebo (van Stegeren et al., 2006), and peripheral infusion of NA resulted in significantly increased sAA levels compared to saline (Kuebler et al., 2014). Further, associations between sAA and plasma levels of NA have also been shown (Thoma et al., 2012).

Central and peripheral levels of NA and adrenaline appear to exhibit diurnal profiles similar to waking performance (Bellesi et al., 2016; Candito et al., 1992). Interestingly, it has been reported that sAA measures also exhibit a similar diurnal profile, with levels peaking in the afternoon and declining towards the evening (Nater et al., 2004; Nater et al., 2007). Additionally, increased sAA activity has also been reported following acute sleep restriction (O'Leary et al., 2015) and total sleep deprivation (Seugnet et al., 2006), suggesting sAA may be a measure of increased sleep pressure. Based on these findings, it is conceivable that changes in sAA may reflect deviations in performance during extended wakefulness. However, few studies have attempted to investigate the association between sAA and performance, particularly during sleep deprivation when sleepiness and performance deficits are increased (Van Dongen et al., 2003).

Under normal sleep-wake conditions, elevated sAA levels have been associated with driver stress (Yamaguchi et al., 2006; Yamaguchi and Sakakima, 2007) and slower reaction times (Muehlhan et

al., 2013). Of the few studies that have explored sAA and performance during sleep deprivation, similarities between sAA secretion and performance have been observed, but not directly investigated (Bachmann et al., 2012; Figueiro and Rea, 2011). Although, Bachmann et al. (2012) observed significantly greater sAA levels in individuals with a genetic mutation associated with increased sleep pressure and impaired vigilance during sleep deprivation.

In the current study, we investigated the potential of sAA as a non-invasive physiological measure of performance during two days of total sleep deprivation. We hypothesized that (1) the diurnal profile of sAA levels would reflect the temporal profile of performance during sustained wakefulness, and (2) sAA levels on Day 2 of sleep deprivation would be increase compared to Day 1, which would be associated with greater performance deficits on Day 2. We addressed these hypotheses by investigating the diurnal profile of sAA levels during two days (50 h) of total sleep deprivation, and exploring the relationship between changes in sAA levels and performance on a psychomotor vigilance task (PVT) and simulated driving task. The simulated driving task was used as a measure of operator performance as the operation of a motor vehicle during continuous wakefulness of 36 – 50 h is common in a range of applied domains including the military (Lieberman et al., 2005) and medical professions (Barger et al., 2005). The PVT was included as it is a validated measure of sustained attention, and sensitive to the effects of both the diurnal rhythm for arousal and the homeostatic pressure for sleep (Lim and Dinges, 2008).

2. Materials and Methods

2.1. Participants

Twelve healthy, non-smoking adults (5 females, 7 males) between the ages of 19 and 28 years (mean $\pm$ SD: 22.5 ± 2.5 years) were selected to participate in the study after undergoing a telephone interview and two physical in-lab screening sessions. All participants were physically and psychologically healthy, as assessed by toxicology screening and questionnaires, and free from any medication (oral contraceptive use permitted ($n = 2$)) and illicit drug use. Participants were low-moderate consumers of alcohol (<7 standard drinks/week) and caffeine (<300 mg/day), and abstained from both in the week prior to the study. Trans-meridian travel or shift work during the three months

prior to taking part in the study was exclusionary. All participants exhibited regular sleep patterns with an average bedtime before 00:00h ($23:48\text{h} \pm 00:44\text{h}$), wake-up time before 09:00h ($08:13\text{h} \pm 00:37\text{h}$), and 7 – 8 h of total sleep time ($7.4 \pm 0.87\text{ h}$) per night. Two weeks prior to taking part in the study, daily sleep and wake times were documented in self-reported sleep logs and verified with activity monitors (Actiwatch 2, Philips Respironics, Oregon, USA). One female participant was excluded from analysis after completion of study due to missing saliva samples. Participant demographics ($n = 11$) are presented in Table 1.

This study was approved by the University of South Australia Human Ethics Research Committee and carried out in accordance with the guidelines for human research established by the National Health and Medical Research Council of Australia. All participants gave written informed consent prior to being enrolled in the study, and were made aware their participation was completely voluntary and that they could withdraw at any time. Participants were financially compensated for their contribution upon completion of the study.

2.2. Study design

The study was carried out at the Centre for Sleep Research at the University of South Australia. Participants were recruited to take part in a study investigating the effects of caffeine gum administration on performance and sleepiness during 50 h of total sleep deprivation (TSD). Data pertaining to the scope of the larger study are published elsewhere (Johnson et al., 2016; Paech et al., 2016). Only data relating to the control condition (i.e. placebo) are reported here.

Participants entered the sleep laboratory at 12:00h on Day 0 and remained in the laboratory, free from time or social cues, until completion of the study protocol at 21:00h on Day 3 (Fig. 1). Participants completed the four-day study protocol in groups of four, with each assigned an individual bedroom and testing suite. On Day 0, participants familiarized themselves with the sleep laboratory and assessment tasks before being given a 10 h sleep opportunity from 22:00h on Day 0 to 08:00h on Day 1 (Fig. 1). The 10 h baseline sleep opportunity was included to ensure the participants had sufficient sleep ($\geq 8\text{ h}$) prior to the start of the scheduled sleep deprivation period.

Upon awakening from the baseline sleep at 08:00h on Day 1, participants underwent a total of 50 h of TSD beginning at 08:00h on Day 1 and concluding at 10:00h on Day 3 (Fig. 1). At regular 3 h intervals throughout the period of TSD, participants rated their subjective sleepiness, completed a brief 3-min psychomotor vigilance task (PVT-B), performed a 40-min simulated driving task, and collected saliva samples (Fig. 1). Saliva samples were collected within the hour prior to each performance assessment, except pertaining to the first performance at 09:00h on Day 1 (Fig. 1). During free-time participants could watch DVDs, play games (board or card), socialize amongst themselves or with staff members, listen to music, or read books. Participants were instructed to refrain from any strenuous physical activity throughout the protocol, although casual walking within the laboratory was permitted if they were struggling to remain awake. All meals (breakfast, lunch, dinner, and snack) were consumed in a shared area of the laboratory at 07:00-08:00h, 13:00h, 19:00h, and 01:00h.

Although participants remained awake for a total of 50 h, performance assessments and saliva samples were only collected until 46 h of wakefulness, as sufficient time prior to the recovery sleep was required for the application of EEG electrodes and pre-sleep procedures. At the conclusion of the 50 h of continuous wakefulness at 10:00h on Day 3, participants were given a 9 h daytime recovery sleep opportunity (10:00h to 19:00h) prior to leaving the sleep laboratory (Fig. 1). Throughout the study, light intensity at angle of gaze was <50 lux during wake period and <0.03 lux (i.e. complete darkness) during scheduled sleep opportunities, and ambient temperature was $23 \pm 1^{\circ}\text{C}$.

2.3. Salivary α -amylase analysis

To account for diurnal variation in sAA measures (Nater et al., 2004; Nater et al., 2007), saliva samples were categorized into study days (Day 1 and Day 2), with ‘Day 1’ comprised of data collected from 08:00h on Day 1 to 08:00h on Day 2, and ‘Day 2’ consisting of data collected from 08:00h on Day 2 to 08:00h on Day 3 (Fig. 1).

2.3.1. Saliva collection

Saliva samples were collected within the hour preceding each performance assessment, except in the case of the first performance session at 09:00h on Day 1 (Fig. 1). Saliva was not collected within 60-min of awakening from baseline sleep (Fig. 1) due the potential confounding factors associated with the awakening response of sAA (Nater et al., 2007) and the meal at 08:30h.

Saliva was collected using the Salivette[®] (Sarstedt, Numbrecht, Germany) with cotton swab inserts. Participants were instructed to gently chew on the cotton swab insert for 2-min. Salivettes[®] were centrifuged at 1,000 x g for 5-min, aliquoted into Eppendorf[®] (Sigma-Aldrich, NSW, Australia) tubes and stored at -80°C prior to analysis.

2.3.2. Assay procedure

Biochemical analyses were performed at the School of Pharmacy and Medical Sciences at the University of South Australia. Saliva samples were assayed using a commercially available sAA-specific kinetic enzyme assay kit (Salimetrics[®], State College, PA, USA). On the day of analysis, saliva samples were completely thawed at room temperature prior to being diluted 1:200 with commercially-supplied diluent. Diluted samples (and controls) were added to a 96-well microtiter plate in duplicate, followed by the addition of pre-heated (37°C) substrate solution. The microtiter plate was then warmed in a pre-heated (37°C) plate reader for 60-sec. An initial measurement of optical density at 405nm was recorded at 1-min and then again at 3-min.

sAA activity was determined by subtracting the 1-min reading from the 3-min reading and multiplying by the conversion factor supplied by the manufacturer. To reduce error variance, all samples of a single participant were analyzed in the same experimental run.

2.4. Subjective sleepiness and performance

Participants completed a total of 15 performance assessments during wakefulness (Fig 1). Performance assessments began at 09:00h on Day 1 and occurred at regular 3 h intervals throughout wakefulness, except pertaining to the first two sessions on Day 1, which were 6 h apart as sufficient time was required for intravenous cannulation (part of the larger study).

Data were categorized into study days in the same manner as saliva samples (described in section 2.3), thus each performance assessment coincided with a saliva sample.

2.4.1. Subjective sleepiness

The subjective sleepiness scale was administered on the same hand-held device as the PVT-B (described in section 2.4.2). Participants recorded their subjective sleepiness on a 10-point Likert-type scale ranging from 1 = 'no' to 10 = 'yes' by using the buttons on the device to move the indicator along the scale on the screen. Sleepiness was recorded prior to the beginning of the PVT-B. Data were stored as units between 1 and 10.

2.4.2. PVT-B

The 3-min PVT-B is a validated shorter version (Basner et al., 2011) of the standard 10-min PVT (Dinges and Powell, 1985) performed on a hand-held device (PVT-192; Ambulatory Monitoring Inc., New York, USA). The PVT was included as a measure of performance because it is sensitive to both the diurnal drive for alertness and the homeostatic pressure for sleep (Lim and Dinges, 2008). Participants were required to respond as quickly as possible to the visual stimulus (millisecond counter) presented on the screen of the hand-held device by pressing either the left or right button on the device with their dominant thumb. The visual stimulus was presented at random intervals of 1- to 4-sec. The output measures included the reciprocal-transform of mean reaction time (RT) and mean slowest 10% RT (i.e. mean of the slowest 10% RTs during task).

2.4.3. Simulated driving task

Each participant was assigned a single simulated vehicle station, which consisted of a computer monitor screen with a virtual 120° view, a steering wheel, control pedals, an adjustable driver's seat mounted on motion actuators, and a headset (as described by Stokes et al., 2015). The simulated driving task was included as a measure of performance because the operation of a motor vehicle while sleep deprived or during periods of extended wakefulness is common in sustained operations such as the military (Lieberman et al., 2005) or medical professions (Barger et al., 2005).

The simulated driving task (Virtual Battlespace 2 software (VBS-2); Bohemia Interactive Simulations, Florida, USA) mimicked a 40-min rural highway drive at dusk along a slightly curved road, with no other vehicles, intersections, or obstacles. Participants were instructed to remain in the left-hand lane (in accordance with the Australian road regulations) and maintain a speed of 80 km/h at all times. In the event that the vehicle veered off the road, the simulator reset to a stationary position in the center of the road, and participants were required to press the ignition button on the steering wheel to resume the task. During the task, if there was no response for a period of 10-sec (i.e. vehicle at standstill) a wake-up tone (i.e. loud bell ringing) was played through the headset. If the participant did not respond to three concurrent wake-up tones, research staff intervened to gently wake the participant and encourage them to complete the task. Outcome measures of performance included speed deviation; defined as the standard deviation of the average speed during the 40-min task (excluding period of acceleration from 0 to 80 km/h at start of task or following restarts), and lane deviation; the average deviation of the geometrical center of the vehicle from the center of the road (excluding events where the vehicle veered off the road).

2.4.4. Statistical analyses

Due to positively skewed distributions, sAA, speed deviation, and lane deviation were log-transformed to approximate normal distributions. All statistical analyses were performed using Stata v.14 software (StataCorp, Texas, USA). All mixed-effects models were fit using the maximum likelihood estimation (MLE) method.

To investigate the best model of fit for each variable (sAA levels, subjective sleepiness and performance outcomes), mixed-effects linear models were initially tested and fit diagnostics compared with separate mixed-effects quadratic growth curve models. Model fit was investigated by comparison of the log likelihood and Akaike's information criterion (AIC) (Johnson et al., 2013). The log likelihood is a measure of unexplained variation in the fitted model and the AIC is a measure of 'goodness-of-fit' that takes into account the number of parameters estimated. Smaller values (closer to zero) indicate a better fit of the data. The likelihood-ratio test was performed to investigate whether

the observed differences between models were statistically significant. The significance of quadratic terms in the quadratic growth curve models were determined using the Wald's test.

For all mixed-effects models, the linear fixed-effect of 'Day' (centered at Day 1) represents the average change from Day 1 to Day 2, and the linear (Time) and quadratic (Time²) fixed-effects of time of day (centered at 08:00h for sAA measures and 09:00h for performance outcomes) represent the instantaneous and overall trend across time awake, respectively. The random-effects of the intercept and the residual accounted for variability between- and within-persons, respectively.

To investigate the association between sAA measures and performance during wakefulness, separate mixed-effects models performed with fixed-effects of sAA measures as predictors of performance variables. The proportion of the variance in the model (i.e. effect size) explained by sAA measures was calculated according to the guidelines outlined by Kristjansson et al. (2007) for mixed-effects modelling. Data are reported as estimated coefficient $\pm$ standard error (SE) with effect size (ES).

All graphs are estimated marginal means (i.e. fitted values) $\pm$ 95% confidence intervals (CI) of the best-fit mixed-effects model, unless indicated otherwise. Significance was assumed at $p < 0.05$. All data reported are mean $\pm$ SE, unless indicated otherwise.

3. Results

3.1. Sustained attention and driving performance show diurnal variation during TSD

3.1.1. PVT-B performance

Results from the mixed-effects linear and quadratic growth curve models of mean RT and mean slowest 10% RT are presented in Table 2. The inclusion of a quadratic polynomials (Time² and Time² X Day) significantly improved the fit of the model for PVT-B performance data (mean RT: $\chi^2(2) = 44.2, p < 0.001$; mean slowest 10% RT: $\chi^2(2) = 53.0, p < 0.001$) (Table 2).

During Day 1, there was no significant change in RT from 09:00h (Time: mean RT, $p = 0.26$; mean slowest 10% RT, $p = 0.06$), suggesting that PVT-B performance was stable during the baseline waking-day (Fig. 2a and 2b). The negative coefficient of the quadratic term for time of day indicated a

small, but significant, decrease in reciprocal-transform of RT as wakefulness increased towards the evening and early-morning of Day 1 (Table 2) (Time²: mean RT, $p = 0.001$; mean slowest 10% RT, $p < 0.001$). Data are reciprocal-transform of mean RT and mean slowest 10% RT, thus a decrease indicates worse performance (Fig. 2a and 2b).

As wakefulness continued into Day 2, there was a significant increase in reciprocal-transform of RT (i.e. better performance) from 09:00h (Day X Time: mean RT and mean slowest 10% RT, $p = 0.003$), with performance peaking in the afternoon (between 15:00h and 18:00h). However, similar to the profile pattern during Day 1, the negative coefficient of the Day X Time² interaction indicated a steady decline in mean RT and mean slowest 10% RT as wakefulness increased into the evening and early-morning of Day 2 (Table 2) (Day X Time²: mean RT, $p = 0.031$; mean slowest 10% RT, $p = 0.009$) (Fig. 2a and 2b).

Despite the slight improvement in performance during the afternoon of Day 2, PVT-B performance was significantly worse on Day 2 as compared to Day 1 (Day: mean RT and mean slowest 10% RT, $p < 0.001$) (Table 2).

3.1.2. Simulated driving performance

The results of the mixed-effects models linear and quadratic growth curve models of log-transformed driving performance measures are presented in Table 2, while anti-logged values are presented in Fig. 2c and 2d to facilitate interpretation. The inclusion of a quadratic polynomials (Time² and Day X Time²) significantly improved the fit of the model for driving performance data (speed deviation: $\chi^2(2) = 76.5$, $p < 0.001$; lane deviation: $\chi^2(2) = 26.7$, $p < 0.001$) (Table 2).

Driving performance measures demonstrated significant diurnal profiles during sleep deprivation (Fig. 2c and 2d). During Day 1, there was a decrease in speed and lane deviation from the first driving assessment at 09:00h (Time: speed deviation, $p < 0.001$; lane deviation, $p = 0.02$), indicating an improvement in driving performance during the waking-day (Table 2) (Fig. 2c and 2d). The positive coefficient of the quadratic term (Time²) indicated a small, but significant, increase in speed and lane

deviation (i.e. worse performance) as wakefulness increased towards the evening and early-morning (Time²: speed deviation and lane deviation, $p < 0.001$) (Table 2) (Fig. 2c and 2d).

As wakefulness continued into Day 2, the diurnal profile did not change as compared to Day 1 (day X time, speed deviation: $p = 0.65$; lane deviation: $p = 0.33$) (day X time², speed deviation: $p = 0.28$; lane deviation: $p = 0.73$) (Table 2) (Fig. 2c and 2d). Speed and lane deviation decreased during the waking-day (i.e. performance improved) before steadily increasing (i.e. performance decreased) towards the evening and early-morning (Fig. 2c and 2d). Similar to PVT-B performance, driving performance peaked at approximately 15:00h and was significantly worse on Day 2 of sleep deprivation as compared to Day 1 (Day: speed deviation and lane deviation, $p < 0.001$) (Table 2) (Fig. 2c and 2d).

3.2. Subjective sleepiness ratings demonstrate constant linear increase during TSD

Results of mixed-effects linear and quadratic growth curve models are presented in Table 3. The inclusion of a quadratic polynomials (Time² and Day X Time²) were not significant (Time², $p = 0.21$; Day X Time², $p = 0.75$) and did not improve model fit ($\chi^2(2) = 2.48$, $p = 0.29$) (Table 3).

In contrast to performance, ratings of subjective sleepiness demonstrated a constant linear increase during sleep deprivation (Time, $p < 0.001$) (Fig. 2e). The rate of increase in sleepiness was significantly lower during day 2 as compared with day 1 (Day X Time; $p = 0.001$) (Table 3).

3.3. Diurnal profile of sAA coincides with performance during wakefulness

Similar to PVT-B and driving performance, sAA measures demonstrated a marked diurnal profile across the two days of TSD (Fig. 2f). Results of the mixed-effects linear and quadratic growth curve models of log-transformed sAA values are presented in Table 3. Inclusion of a quadratic polynomials (Time² and Day X Time²), and removal of non-significant linear interaction term (Day X Time, $p = 0.092$), significantly improved the fit of the model for log-transformed sAA levels ($\chi^2(1) = 48.5$, $p < 0.001$) (Table 3).

Similar to performance, sAA values increased during the waking-day (Time, $p < 0.001$), peaked in the afternoon at approximately 15:00h, and steadily declined as wakefulness continued towards the evening and early-morning (Time², $p < 0.001$) (Fig. 2f; Table 3). This diurnal profile did not significantly change as wakefulness continued into Day 2 (Day X Time², $p = 0.85$) (Table 3), and there was no significant difference in sAA levels on Day 2 compared to Day 1 (Day, $p = 0.06$) (Fig. 2f; Table 3).

3.3.1. Association with PVT-B performance

Separate mixed model analysis revealed that log-transformed sAA levels were positively associated with mean RT (0.1867 (0.0784), $p = 0.017$, ES = -0.26) and mean slowest 10% RT (0.2707 (0.1113), $p = 0.015$; ES = -0.37), indicating higher log-transformed sAA values were associated with an increase in the reciprocal-transform of RT (i.e. better performance).

To illustrate the association between sAA and performance, fitted values derived from mixed-effects quadratic growth curve model for PVT-B performance measures (reported in section 3.1.1) are presented together with the fitted values derived from mixed-effects quadratic growth curve model for log-transformed sAA levels (reported in section 3.3) in Fig. 3a and 3b (left panel). The diurnal profile of log-transformed sAA levels closely tracked with PVT-B performance during Day 1 (Fig. 3a and 3b, left panel). However, sAA levels appeared to be higher than PVT-B measures during Day 2 of wakefulness (Fig. 3a and 3b, left panel). Further analysis revealed that the average change from Day 1 to Day 2 for mean RT and mean slowest 10% RT was equivalent to 1.8 and 2.6 log-transformed units of sAA, respectively (Fig. 3a and 3b, left panel). Thus, sAA levels were adjusted accordingly (i.e. sAA levels on Day 2 reduced by 1.8 units for mean RT and 2.6 units for mean slowest 10% RT), which resulted in a better indication of PVT-B performance by sAA values during Day 2 of wakefulness (Fig. 3a and 3b, right panel).

3.3.2. Association with simulated driving performance

Separate mixed model analysis revealed that log-transformed sAA levels were negatively associated with speed deviation (-0.1444 (0.0624), $p = 0.021$; ES = -0.27) and lane deviation (-0.1198 (0.0319), p

<0.001 ; $ES = 0.45$), suggesting that higher sAA values were associated with decreased speed and lane deviation (i.e. better performance).

Fitted values derived from mixed-effects quadratic growth curve models of log-transformed speed deviation and lane deviation (reported in section 3.1.2) are presented together with the fitted values derived from mixed-effects quadratic growth curve model of log-transformed sAA levels (reported in section 3.3) in Fig. 3c and 3d (left panel). Similar to PVT-B performance, the diurnal profile of log-transformed sAA levels mirrored driving performance during Day 1 (Fig. 3c and 3d, left panel), however, sAA levels were higher than speed deviation and lane deviation during Day 2 of wakefulness. Further analysis revealed that the average change from Day 1 to Day 2 for log-transformed speed deviation and lane deviation was equivalent to 2.2 and 2.6 log-transformed units of sAA, respectively (Fig. 3c and 3d, left panel). Thus, sAA levels were adjusted accordingly (i.e. sAA levels on Day 2 reduced by 2.2 units for speed deviation and 2.6 units for lane deviation), resulting in sAA values closely tracking driving performance during Day 2 of wakefulness (Fig. 3c and 3d, right panel).

4. Discussion

In the current study, we provide further evidence of discrepancies between subjective feelings of sleepiness and objective performance, and demonstrate the potential of sAA to be considered as a candidate marker for physiological monitoring of operator performance during sustained wakefulness. Our results show that during two days of sleep deprivation, ratings of subjective sleepiness demonstrated a constant linear increase, which did not coincide with the diurnal profile of PVT-B and driving performance measures. In contrast, sAA values demonstrated a marked diurnal profile similar to performance, with levels peaking in the afternoon and declining in the evening and early-morning. Furthermore, a significant association between sAA measures and performance was revealed, with higher sAA levels associated with better performance, and a decline in sAA levels associated with greater performance deficits. Additionally, we also demonstrated that although the diurnal profile of sAA did not change during Day 2 of wakefulness, as it did with performance, adjusting sAA levels by

a constant factor relative to the change in performance between study days, resulted in sAA levels that closely tracked performance during Day 2 of sleep deprivation. Taken together, our findings highlight the potential of sAA to be further investigated as a candidate marker of operator performance during extended wakefulness.

Sleep deprivation as a result of extended work shifts causes increased sleepiness and significant performance impairments (Johnson et al., 2016; Paech et al., 2016; Van Dongen et al., 2003). However, evidence of inter-individual differences in performance during sleep deprivation (Van Dongen et al., 2004), suggest that operator performance is not solely influenced by the extent of sleepiness. Waking performance is mediated by the additive interaction of the increased homeostatic pressure for sleep and the 24 h diurnal rhythm for arousal and alertness (Achermann and Borbely, 1994; Dijk et al., 1992; Van Dongen and Dinges, 2003). Thus, a suitable physiological measure of operator performance should be sensitive to both processes in order to reliably reflect an individual's capacity to perform a task; rather than merely monitor how sleepy they are feeling at the time (Balkin, 2011). Our findings of similar diurnal profiles between sAA and performance during two days of extended wakefulness, suggest that sAA may be sensitive to the diurnal rhythm for arousal and monitor performance by closely tracking the diurnal drive for alertness. The diurnal variation of demonstrated by sAA measures (Nater et al., 2004; Nater et al., 2007) and performance (Lim and Dinges, 2008) observed in the current study are consistent with previous reports, and reminiscent of the diurnal rhythm of arousal driven by central noradrenergic mechanisms (Chamberlain and Robbins, 2013). Thus, it is plausible that the association between sAA and performance may be explained by the underlying role of NA in sAA secretion and performance. Recently, Bellesi et al. (2016) used a mouse model to demonstrate that cognitive impairments during sleep deprivation were associated with diurnal changes in cortical NA levels. Similarly, blockade of cortical NA re-uptake sites (Robinson, 2012) and increased thalamic activity driven by central NA (Chee et al., 2008) have been associated with improved accuracy on RT tasks. These reports are consistent with our own findings that higher sAA levels are associated with better RT and simulated driving performance, and similar to findings by Muehlhan et al. (2013) under normal sleep-wake conditions.

Unlike performance, which was significantly worse on Day 2 of wakefulness compared to Day 1, sAA levels were not significantly different on Day 2 compared to Day 1. These findings are not consistent with previous reports proposing sAA as a measure of increased sleep pressure (Bachmann et al., 2012; O'Leary et al., 2015; Seugnet et al., 2006). These conflicting results with our own findings could be explained by individual variability in sAA activity following sleep deprivation (Bachmann et al., 2012; Nater et al., 2004; Seugnet et al., 2006). Thus, it may be for some individuals that relatively higher sAA levels attenuate the usual decline in performance during sleep loss. In addition, inconsistencies between our own findings and previous literature may be explained by less frequent saliva sampling in previous studies where saliva was only collected at a single time point at baseline and after sleep manipulation. Also, performance was either not investigated in these studies or a different assessment task was used to investigate performance deficits. Our findings suggest that sAA may not be sensitive to both modulators of performance (i.e. diurnal rhythm for arousal and homeostatic pressure for sleep) during two days of acute TSD as hypothesized. However, our results are limited to the effects of only two days of sleep deprivation. Thus, it might be that a greater period of continuous wakefulness (> 50 h), which is not uncommon in the military (Lieberman et al., 2005), is required to disrupt the robust diurnal profile of sAA secretion. Nevertheless, findings that the severity of performance impairments does not significantly increase from 51 h to 75 h of wakefulness (Killgore et al., 2007), suggest that adjusting sAA levels by a constant factor relative to the average change in performance during 50 h of TSD may be suitable. These observations highlight the appeal in further investigation of sAA as a physiological measure of performance during extended wakefulness.

To our knowledge, this is the first study to report a diurnal profile in sAA secretion beyond 24 h of continuous wakefulness, and demonstrate a significant association between sAA and simulated driving performance, as well as RT, during two days of sleep deprivation. This study was conducted in a laboratory-controlled environment, and in a relatively small sample of non-shift working, healthy adults. For our findings to translate into real-world settings, further investigation would be required in a larger sample size and in individuals who may be chronically sleep restricted or adapted to the

effects of sustained wakefulness. Further, it would also be of interest to explore the effects beyond 50 h of sustained wakefulness on sAA levels and performance to examine if the homeostatic pressure for sleep eventually overrides the robust diurnal rhythm in sAA activity. Future work may also involve exploring our findings using different techniques for saliva collection. There is an ongoing discussion about the reliability of sAA measures in research due to the confounding factors associated with saliva collection via the Salivette[®] technique employed in the present study and salivary flow rate on sAA secretion (see Bosch et al., 2011). However, stress-induced sAA increases independent of salivary flow rate have been reported in a study comparing the passive drool technique and the Salivette[®] method (Rohleder et al., 2006). Future research may also involve consideration of other subjective sleepiness scales such as the Karolinska Sleepiness Scale (KSS) or Stanford Sleepiness Scale (SSS), as the disparity between subjective sleepiness and objective performance in the current study may be influenced by the type of sleepiness scale implemented. The 10-point Likert-type scale is not as well validated in research and clinical practice as the KSS or SSS (see Shahid et al., 2010), although our group has recently demonstrated similar patterns between the 10-point Likert-type scale and the KSS (Paech et al., 2016).

The strengths of our study include the period of sleep deprivation, which is common in sustained operations such as the military (Lieberman et al., 2005) and medical professions (Barger et al., 2005). Although, our findings are also limited to only this period of TSD and future work requires exploring these findings during sleep restriction, which is common to the wider community and not specific to sustained operations. However, there is evidence suggesting that chronic restriction of sleep to less than 6 h per night produces cognitive impairments equivalent to the effects of up to two night of TSD (Van Dongen et al., 2003). These reports suggest that our findings may be extended to sleeping patterns more commonly experienced in everyday life. The regular sampling of saliva and performance assessment are also a major strength of this study, as frequent and equally distributed sampling allows us to explore the circadian influence on variables measured.

Validation of the findings in this study could be useful in establishing individually-tailored working schedules or assessing 'fitness-for-duty' in sustained operations in a non-invasive, non-obtrusive, and

objective manner. For example, if an individual exhibits significant decreases in sAA levels following acute total sleep loss then greater performance deficits could be anticipated and these individuals could be scheduled for work appropriately. These findings are particularly important as inter-individual difference in performance (Van Dongen et al., 2004) emphasize the need for individual-specific measures of performance capability. Furthermore, the recent development of a hand-held sAA analyzer (Shetty et al., 2011), highlights the appeal in investigating the potential of sAA as a measure for physiological monitoring of performance during sustained operations and in research. sAA assessment may also be useful in establishing ideal times for administration of effective countermeasures such as caffeine or modafinil (Paech et al., 2016; Winder-Rhodes et al., 2010). There is evidence to suggest that these attention-modulating stimulants act by counteracting reduction in central NA associated with extended wakefulness (Smith et al., 2003) or activating noradrenergic neurons in the LC (Hou et al., 2005), and their use has been linked with changes in sAA (Klein et al., 2010; Winder-Rhodes et al., 2010).

Our findings highlight the potential for sAA to be investigated further as a candidate marker of physiological monitoring of performance during extended wakefulness. We report that a decline in sAA during the evening coincided with a decline in performance, while higher sAA levels during the waking-day were associated with better performance. We also show that adjusting sAA levels during Day 2 of TSD (i.e. > 24 h of extended wakefulness) by a constant factor relative to the average change in performance resulted in sAA levels closely tracking performance at times when the increased homeostatic pressure for sleep did not impact sAA to the same extent as it did performance. The non-invasive and simple handling of saliva collection appeals to the utility of salivary assessment in operation environments and in research. Our findings highlight the potential of sAA to be considered as a candidate marker for physiological monitoring of performance during sleep deprivation.

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Conflict of interest

None

Contributors

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Table 1: Participant demographic information ($n = 11$).

	Mean $\pm$ SD	Range
Age (years)	22.3 $\pm$ 2.5	19 – 28
Gender (% male)	63.6%	4 Female; 7 Male
Body Mass Index (BMI) (kg/m ²)	22.4 $\pm$ 2.1	19.4 – 26.9
Habitual Wake-Time (hh:mm)	08:13 $\pm$ 0.37 min	07:38 – 09:26
Habitual Bed-Time (hh:mm)	23:48 $\pm$ 0.44 min	22:31 – 00:34
Total Sleep Time (h)	7.4 $\pm$ 0.9	6.5 – 8.8
Habitual caffeine (cups/day)	1.5 $\pm$ 1.5	0.0 – 5.0
Habitual alcohol (standard drinks/ week)	2.0 $\pm$ 1.7	0.0 – 4.0

Table 2: Mixed-effects linear (model 1) and quadratic growth curve (model 2) models on reciprocal-transform of mean RT and mean slowest 10% RT, and log-transformed speed deviation and lane deviation during two days of sleep deprivation.

	Mean RT				Mean slowest 10% RT				Speed deviation				Lane deviation			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
	Coeff. (SE)	<i>p</i> <i>value</i>	Coeff. (SE)	<i>p</i> <i>value</i>	Coeff. (SE)	<i>p</i> <i>value</i>	Coeff. (SE)	<i>p</i> <i>value</i>	Coeff. (SE)	<i>p</i> <i>value</i>	Coeff. (SE)	<i>p</i> <i>value</i>	Coeff. (SE)	<i>p</i> <i>value</i>	Coeff. (SE)	<i>p</i> <i>value</i>
Intercept	4.907 (0.157)	<0.001	4.695 (0.164)	<0.001	3.306 (0.213)	<0.001	2.973 (0.221)	<0.001	0.807 (0.108)	<0.001	1.180 (0.111)	<0.001	-0.556 (0.066)	<0.001	-0.362 (0.072)	<0.001
Time	-0.129 (0.022)	<0.001	0.072 (0.064)	0.258	-0.142 (0.033)	<0.001	0.174 (0.094)	0.064	0.166 (0.019)	<0.001	-0.189 (0.054)	<0.001	0.094 (0.013)	<0.001	-0.091 (0.038)	0.016
Day	-0.996 (0.128)	<0.001	-1.167 (0.142)	<0.001	-1.120 (0.194)	<0.001	-1.417 (0.208)	<0.001	1.676 (0.114)	<0.001	1.575 (0.119)	<0.001	0.943 (0.075)	<0.001	0.913 (0.083)	<0.001
Day X Time	0.085 (0.030)	0.004	0.267 (0.090)	0.003	0.069 (0.045)	0.126	0.388 (0.132)	0.003	-0.117 (0.026)	<0.001	-0.036 (0.075)	0.653	-0.071 (0.017)	<0.001	-0.052 (0.053)	0.327
Time ²			-0.028 (0.009)	0.001			-0.045 (0.013)	<0.001			0.050 (0.007)	<0.001			0.026 (0.005)	<0.001
Day X Time ²			-0.026 (0.012)	0.031			-0.047 (0.018)	0.009			-0.011 (0.010)	0.282			-0.002 (0.007)	0.734
AIC	229.2		189.0		359.6		310.6		180.8		122.6		37.57		-0.337	

Time: Linear term; Time²: Quadratic term.

Time = centered at 09:00h, increasing by 1 every 3 h.

Day = centered at Day 1.

Coeff. = Fixed-effects coefficient

AIC = Akaike's information criterion

Table 3: Mixed-effects linear (model 1) and quadratic growth curve (model 2) models of subjective sleepiness and log-transformed sAA levels.

	Subjective Sleepiness				sAA			
	Model 1		Model 2		Model 1		Model 2	
	Coeff. (SE)	<i>p value</i>	Coeff. (SE)	<i>p value</i>	Coeff. (SE)	<i>p value</i>	Coeff. (SE)	<i>p value</i>
Intercept	2.253 (0.649)	0.001	2.499 (0.706)	<0.001	5.085 (0.259)	<0.001	4.522 (0.258)	<0.001
Time	0.598 (0.083)	<0.001	0.364 (0.281)	0.194	-0.153 (0.021)	<0.001	0.218 (0.046)	<0.001
Day	4.019 (0.489)	<0.001	4.122 (0.621)	<0.001	-0.389 (0.120)	0.001	-0.145 (0.078)	0.062
Day X Time	-0.377 (0.113)	0.001	-0.492 (0.393)	0.210	0.047 (0.028)	0.092		
Time ²			0.033 (0.038)	0.384			-0.046 (0.006)	<0.001
Day X Time ²			0.017 (0.053)	0.753			-0.001 (0.003)	0.852
AIC	674.4		675.9		191.1		144.6	

Time: Linear term; Time²: Quadratic term.

Time = centered at 09:00h for sleepiness and 08:00h for sAA, increasing by 1 every 3 h.

Day = centered at Day 1.

Coeff. = Fixed-effects coefficient

AIC = Akaike's information criterion

Fitted Values

(a)

Adjusted Values

(b)

(c)

(d)