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Effects of strategic early-morning caffeine gum administration on association between salivary alpha-amylase and neurobehavioural performance during 50 h of sleep deprivation

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ABSTRACT

Self-assessment is the most common method for monitoring performance and safety in the workplace. However, discrepancies between subjective and objective measures have increased interest in physiological assessment of performance. In a double-blind placebo-controlled study, 23 healthy adults were randomly assigned to either a placebo (n = 11; 5 F, 6 M) or caffeine condition (n = 12; 4 F, 8 M) while undergoing 50 h (i.e. two days) of total sleep deprivation. In previous work, higher salivary alpha-amylase (sAA) levels were associated with improved psychomotor vigilance and simulated driving performance in the placebo condition. In this follow-up article, the effects of strategic caffeine administration on the previously reported diurnal profiles of sAA and performance, and the association between sAA and neurobehavioural performance were investigated. Participants were given a 10 h baseline sleep opportunity (monitored via standard polysomnography techniques) prior to undergoing sleep deprivation (total sleep time: placebo = 8.83 ± 0.48 h; caffeine = 9.01 ± 0.48 h). During sleep deprivation, caffeine gum (200 mg) was administered at 01:00 h, 03:00 h, 05:00 h, and 07:00 h to participants in the caffeine condition (n = 12). This strategic administration of caffeine gum (200 mg) has been shown to be effective at maintaining cognitive performance during extended wakefulness. Saliva samples were collected, and psychomotor vigilance and simulated driving performance assessed at three-hour intervals throughout wakefulness. Caffeine effects on diurnal variability were compared with previously reported findings in the placebo condition (n = 11). The impact of caffeine on the circadian profile of sAA coincided with changes in neurobehavioural performance. Higher sAA levels were associated with improved performance on the psychomotor vigilance test during the first 24 h of wakefulness in the caffeine condition. However, only the association between sAA and response speed (i.e. reciprocal-transform of mean reaction time) was consistent across both days of sleep deprivation. The association between sAA and driving performance was not consistent across both days of sleep deprivation. Results show that the relationship between sAA and reciprocal-transform of mean reaction time on the psychomotor vigilance test persisted in the presence of caffeine, however the association was relatively weaker as compared with the placebo condition.

1. Introduction

Waking performance is mediated by the interaction between the homeostatic pressure for sleep and the 24 h circadian propensity for alertness (Dijk et al., 1992; Van Dongen and Dinges, 2003). The homeostatic pressure for sleep increases with time awake and dissipates during sleep, while the circadian propensity for alertness peaks in the late-afternoon and steadily declines during the evening (Borbely, 1982). A relatively even level of alertness and neurobehavioural performance is maintained during the first 16 h of wakefulness, as the circadian drive for alertness counteracts the increased homeostatic pressure for sleep (Achermann and Borbely, 1994). However, extended

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work shifts into the night result in occupational performance deficits as the circadian propensity for alertness declines and the homeostatic pressure for sleep continues to increase (Dijk et al., 1992; Lim and Dinges, 2008). Sleepiness-induced performance deficits are of serious concern in industries such as the military (Lieberman et al., 2005), emergency services (Barger et al., 2005; Patterson et al., 2012), and transportation (Thompson and Stevenson, 2014), where working during the night or while significantly sleep deprived is common and performance impairments can have life-threatening consequences (Tharion et al., 2003; Patterson et al., 2012).

Self-assessment of sleepiness and performance is the most feasible and easily implemented method for monitoring operator performance and safety in the workplace (Christodoulou, 2012). However, the validity of subjective measures is impacted by inter-individual differences in vulnerability to sleep loss (Van Dongen et al., 2004; Rupp et al., 2012; Goel et al., 2015), and discrepancies with objective measures have been documented (Biggs et al., 2007; Paech et al., 2016; Zhou et al., 2012). These findings have led to an interest in objective physiological monitoring of operator performance. Measures such as EEG recordings (D'rozario et al., 2013), ocular metrics (Jackson et al., 2016), and heart rate variability (Chua et al., 2012) have been identified as potential physiological predictors of sleepiness-induced performance decrements during sleep deprivation. In addition, we recently identified a positive association between higher salivary alpha-amylase (sAA) levels and improved neurobehavioural performance during two days of total sleep deprivation (Pajcin et al., 2017).

Alpha-amylase is one of the principal proteins produced by the salivary glands (Zakowski and Bruns, 1985) and has been proposed as a non-invasive peripheral measure of sympathetic nervous system (SNS) activity (Nater and Rohleder, 2009). In addition, it has also been suggested that sAA may be a potential physiological measure of sleep drive, with studies reporting increased sAA levels following extended wakefulness (Seugnet et al., 2006; Bachmann et al., 2012). We previously showed that sAA exhibited a diurnal profile similar to the circadian propensity for alertness, with a peak in the afternoon and steady decline during the evening and early-morning (Pajcin et al., 2017). Moreover, we reported an association between higher diurnal sAA levels and improved performance on a brief psychomotor vigilance test and simulated driving task during extended wakefulness (Pajcin et al., 2017). In this follow-up article, we investigated whether strategic early-morning caffeine gum administration during two days of total sleep deprivation had an effect on the diurnal profiles of sAA and neurobehavioural performance, and if the association between higher sAA and improved neurobehavioural performance persisted in the presence of caffeine.

Caffeine (1,3,5 – trimethylxanthine) is one of the most widely consumed psychostimulants (Fulgoni et al., 2015) and an effective countermeasure for sleepiness-induced performance impairments during sleep loss (Kamimori et al., 2005, 2015; Johnson et al., 2016; Paech et al., 2016). Caffeine is an adenosine receptor antagonist that affects behavioural and physiological processes by blocking adenosine signalling (Fredholm, 1995). Adenosine is a by-product of the primary cellular energy source, adenosine triphosphate (Dashty, 2013), and has been proposed as a mediator of sleep homeostasis during sleep loss (Porkka-Heiskanen et al., 1997). The antagonistic properties of caffeine suggest that it promotes alertness and improves performance by interacting with the homeostatic modulators that mediate performance and sleep propensity (Wyatt et al., 2004). Studies exploiting this theory have identified that repeated low – moderate doses of caffeine in the early-morning, when the circadian drive for alertness is low and the homeostatic pressure for sleep is high (Zhou et al., 2011), is the most effective way to administer caffeine as a countermeasure for sleepiness-induced performance impairments (Wyatt et al., 2004; Kamimori et al., 2005; Johnson et al., 2016; Paech et al., 2016).

The stimulatory effects of caffeine on alertness could be explained by the increased SNS activity that occurs as a result of caffeine blocking adenosine signalling, thus causing an increase in the release of neurotransmitters such as noradrenaline (NA) (Fredholm et al., 1999; Deurveilher et al., 2006; Smith et al., 2003). Previous studies have shown that arousal and vigilance are modulated by the stimulatory actions of NA (Smith and Nutt, 1996; Smith et al., 2003; Bellesi et al., 2016). Furthermore, NA has a major role in stimulating the secretion of sAA from salivary glands (Nater and Rohleder, 2009). Thus, it is plausible that caffeine may also indirectly increase sAA activity. However, literature regarding the effects of caffeine on sAA activity is limited. Studies of habitual caffeine users have reported no association between caffeine intake and sAA activity (Nater et al., 2007; Klein et al., 2014). In contrast, studies of acute caffeine intake have reported increased sAA activity consistent with the sympathomimetic effects of caffeine. For example, increased sAA levels and blood pressure recordings have been reported following ingestion of caffeinated beverages (Bishop et al., 2006; Lin et al., 2014; Papakonstantinou et al., 2016) and a dose-dependent effect of caffeine on sAA activity has also been documented in habitual caffeine users (Klein et al., 2010). In addition, increased caffeine intake was identified as a significant predictor of elevated sAA levels in registered nurses during patient care (Morrison et al., 2003). Based on these findings it is plausible that caffeine-mediated increases in alertness and cognitive performance may coincide with increased sAA activity.

We previously reported that sAA exhibited a circadian profile similar to the propensity for alertness and relatively higher diurnal sAA levels were associated with improved neurobehavioural performance in the placebo condition during extended wakefulness (Pajcin et al., 2017). Given the popularity of caffeine use as a countermeasure for sleepiness-induced performance impairments (Fulgoni et al., 2015; McLellan et al., 2016), the main objective of the current follow-up article was to investigate whether the previously identified relationship between higher sAA and improved neurobehavioural performance persisted in the presence of early-morning caffeine administration. This strategic administration of caffeine in the early-morning has been shown to effectively mitigate performance deficits during extended wakefulness (Wyatt et al., 2004; Kamimori et al., 2005; Johnson et al., 2016; Paech et al., 2016). It was hypothesised that early-morning repeat dose of caffeine gum would increase sAA levels and attenuate the circadian decline in sAA during the early-morning of sleep deprivation as compared to placebo (i.e. no caffeine gum). Furthermore, elevated sAA would be associated with enhanced psychomotor vigilance and simulated driving performance in the caffeine condition.

2. Methods

The data presented in the current article are part of a larger double-blind placebo-controlled study for which 23 participants were randomly assigned to either a placebo (n = 11; 5 females, 6 males) or caffeine (n = 12; 4 females, 8 males) condition. In our previous work, we identified an association between higher sAA and improved neurobehavioural performance in the placebo condition (Pajcin et al., 2017). In the current article, we investigated the effects of strategic early-morning caffeine gum administration on diurnal trajectories of sAA and performance, in comparison with previous findings in the placebo condition (n = 11), and whether previously reported relationship between sAA and neurobehavioural performance persisted in the caffeine condition (n = 12).

2.1. Participants

Data of both conditions (i.e. caffeine and placebo) were collected from a total of 23 young (aged 18–31 years), non-smoking males (n = 15) and females (n = 8). All participants were psychologically and physiologically healthy, and free from any medication (oral contraceptive use permitted [n = 3]). All participants had a body mass index between 19–27 kg/m^2, reported good quality sleep (e.g. Pittsburgh Sleep Quality Index score < 5 (Buysse et al., 1989)), were neither

extreme morning- or evening-type (e.g. Composite Scale of Morningness score between 23–43 (Smith et al., 1989)), and possessed a valid driver's license or had previous experience driving. Recent illicit drug use (within 3 months of participation), donating blood within 30 days of taking part in the study, previous shift work, history of disrupted sleep (e.g. snoring, frequently waking up to go to bathroom), history of psychiatric or medical illness, and excessive caffeine (> 3 cups/day) or alcohol (> 7 standard drinks/week) consumption were exclusionary. Two weeks prior to taking part in the study, regular sleep-wake patterns were monitored by self-reported sleep logs and wrist actigraphy monitors (Actiwatch 2, Philips Respironics, Oregon, USA). Participants were instructed to maintain sleep-wake schedules with a total sleep time between 7–9 h per night, average bedtime between 22:00–23:00 h, and wake-up time between 08:00– 09:00 h. Irregular sleep-wake patterns (e.g. bedtime after 00:00 h or wake time after 09:00 h) and failure to comply with the pre-study requirements (e.g. failure to wear actigraphy monitor at all times) were also exclusionary.

The study was a double-blind placebo-controlled design, with participants randomly assigned to either a caffeine (n = 12; 4 females, 8 males) or placebo (n = 11; 5 females, 6 males) condition. Data pertaining to the caffeine condition are the primary focus of the current article. Participant demographics of the caffeine condition are presented in Table 1. Data pertaining to the placebo condition are published elsewhere (Pajcin et al., 2017). There were no statistically significant differences between conditions.

This study was approved by the University of South Australia Human Research Ethics Committee and carried out in accordance with the Australian Code for the Responsible Conduct of Research and the National Statement on Ethical Conduct in Human Research established by the National Health and Medical Research Council of Australia and Universities Australia. All participants provided written informed consent prior to taking part in the study. Participants were made aware their participation in the study was completely voluntary and they could withdraw at any time. Participants were financially compensated for their contribution upon completion of the study.

2.2. Study design

The four-day in-lab study protocol (Fig. 1) was conducted in the sleep laboratory at the Centre for Sleep Research at the University of South Australia. Participants completed the study protocol in groups of four, with each group comprised of participants within the same condition (i.e. placebo or caffeine). Participants remained in the sleep laboratory free from any time or social cues for the entire duration of the experimental protocol. The study protocol (Fig. 1) is identical to that published previously (Pajcin et al., 2017), with the exception of the early-morning caffeine gum administration (described in Section 2.2.1).

Participants arrived at the sleep laboratory at 12:00 h on Day 0 (Fig. 1) and were given the opportunity to familiarise themselves with the equipment used for performance testing and laboratory environment. To ensure the participants received sufficient sleep (minimum of 8 h) prior to undergoing the scheduled 50 h of total sleep deprivation, participants were given a 10 h baseline sleep opportunity from 22:00 h on Day 0 until 08:00 h on Day 1 (Fig. 1). Scheduled sleep periods were monitored via standard polysomnography techniques (Rechtschaffen and Kales, 1968). At 08:30 h on Day 1 participants were served breakfast before commencing their first performance assessment at 09:00 h. Each performance assessment consisted of a brief 3 min psychomotor vigilance test (PVT-B) (described in Section 2.4.1) followed by a 40 min simulated driving task (described in Section 2.4.2). Participants completed the performance assessments at regular 3 h intervals throughout the duration of wakefulness, except pertaining to the first two performance assessments, which were 6 h apart as sufficient time was required for intravenous cannulation (part of larger study). Participants also collected saliva samples throughout wakefulness (described in Section 2.3). Caffeine gum (described in Section 2.2.1) was administered at 01:00 h, 03:00 h, 05:00 h, and 07:00 h during Day 1 and Day 2 of sleep deprivation (Fig. 1).

Table 1
Demographics of participants in the caffeine condition.

	Mean ± SD	Range
Age (years)	22.6 ± 3.3	18–31
Gender (% male)	66.7	Females = 4; Males = 8
Body Mass Index (kg/m^2)	21.7 ± 1.5	19.0–24.1
Habitual bedtime (hh:mm)	23:43 ± 34 min	22:51–00:30
Habitual wake time (hh:mm)	08:22 ± 39 min	07:15–09:50
Total sleep time (hours)	7.2 ± 0.9	5.8–7.9
Habitual caffeine (cups/day)	1.4 ± 1.0	0.0–4.0
Habitual alcohol (standard drinks/week)	2.4 ± 2.0	0.0–6.0

When not engaged in performance assessments, participants were free to watch DVDs, play games, listen to music, or read books. Any strenuous physical activity during the study protocol was not permitted, with the exception of casual walking within the laboratory. All meals (breakfast, lunch, dinner, and snack) were prepared by staff members and consumed in a shared area of the laboratory at 07:00–08:00 h, 13:00 h, 19:00 h, and 01:00 h (Fig. 1). Participants were allowed 30 min to consume main meals (i.e. breakfast, lunch and dinner) and 15 min to consume snacks. Light intensity at angle of gaze was < 50 lx during scheduled wake periods and < 0.03 lx (i.e. complete darkness) during scheduled sleep opportunities. Ambient temperature was 23 ± 1 °C throughout the study.

Although participants underwent a total of 50 h of sleep deprivation, data were only collected for 46 h of wakefulness as sufficient time was required for pre-sleep procedures (e.g. application of EEG electrodes) prior to the 9 h daytime recovery sleep opportunity on Day 3 (Fig. 1).

2.2.1. Caffeine administration

Caffeine gum was administered as two pieces of Military Energy Gum© (Marketright Inc., Illinois, USA) at 01:00 h, 03:00 h, 05:00 h and 07:00 h during Day 1 and Day 2 of sleep deprivation (Fig. 1). Caffeine gum contained 100 mg caffeine per piece and participants were instructed to chew two pieces of gum simultaneously for 5 min. Participants in the caffeine condition received caffeine-containing gum, while participants in the placebo condition were administered two pieces of gum that were similar in taste and appearance, but contained no caffeine.

It has been reported that low – moderate doses of caffeine (e.g. < 250 mg) enhance performance and favourable subjective feelings (e.g. elation) in a dose-dependent manner, while high doses (e.g. 500 mg) can lead to unfavourable subjective feelings (e.g. anxiety, irritability) and less performance enhancement (Kaplan et al., 1997). Pharmacokinetic studies have shown that 85% of caffeine in the gum form is released, with a relative bioavailability of 90%, following a 200 mg dose after chewing for 5 min (Kamimori et al., 2002). Further, the rate of absorption (and excretion) is maintained across repeated doses of 200 mg caffeine at two–hour intervals between 00:00–04:00 h (Syed et al., 2005). The bi-hourly administration of 200 mg caffeine gum has also been shown to effectively maintain vigilance to baseline levels during one night of sleep deprivation (Kamimori et al., 2005). In addition, we recently reported that the repeated 200 mg dose of caffeine (i.e. four doses of 200 mg caffeine every 24 h of wakefulness) during the early-morning (between 01:00–07:00 h) is effective at maintaining performance on the PVT and simulated driving task (Johnson et al., 2016; Paech et al., 2016).

2.3. Salivary analysis

To enable comparison between the caffeine and placebo conditions, salivary analysis is identical to that described previously and data were

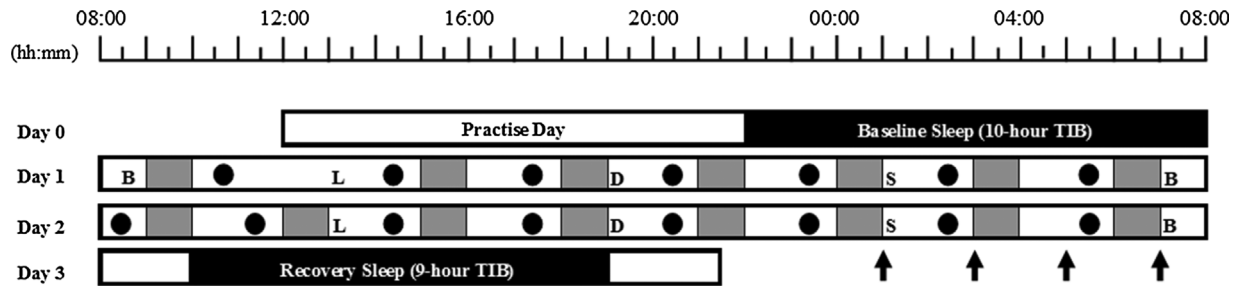


Fig. 1. Four-day in-lab study protocol.
Participants entered the sleep laboratory at 12:00 h on Day 0 and remained in the laboratory, free from time and social cues until conclusion of the experimental protocol at 21:30 h on Day 3. Scheduled sleep opportunities are represented by black bars on Day 0 (10 h time-in-bed (TIB) baseline sleep) and Day 3 (9 h TIB recovery sleep). White bars represent the scheduled 50 h (i.e. two days) of total sleep deprivation, which began at 08:00 h on Day 1 and concluded at 10:00 h on Day 3. During wakefulness, participants collected saliva samples (black circles) and completed performance assessments (grey squares), which consisted of a 3 min psychomotor vigilance test followed by 40 min simulated driving task. Caffeine (200 mg) gum was administered at 01:00 h, 03:00 h, 05:00 h, and 07:00 h (black arrows) during Day 1 and Day 2 of wakefulness. All meals were served at 07:00–08:00 h, 13:00 h, 19:00 h, and 01:00 h (B = breakfast; L = lunch; D = dinner; S = snack). Horizontal axis at the top of figure represents 24 h clock-time (hh:mm).

categorised into two study days as previously reported (Pajcin et al., 2017). Data categorised into Day 1 consisted of data collected from 08:00 h on Day 1 until 08:00 on Day 2 (Fig. 1), and Day 2 consisted of data collected from 08:00 h on Day 2 until 08:00 h on Day 3 (Fig. 1).

Saliva samples were collected via gentle chewing on cotton-swab Salivettes® (Sarstedt, Numbrecht, Germany) for 2 min within the hour preceding each performance assessment (Fig. 1). Salivettes® were centrifuged at 1000 × *g* for 5 min and aliquots stored at −80 °C prior to analysis. Saliva samples were not collected within 60 min of awakening from the 10 h baseline sleep (Fig. 1) because of the potential confounding factors associated with the awakening response of sAA (Nater et al., 2007) and the meal at 08:30 h.

sAA levels were analysed using a commercial sAA-specific kinetic enzyme assay kit (Salimetrics®, State College, PA, USA).

2.4. Performance assessments

Performance data collection is identical to that previously described (Pajcin et al., 2017). PVT-B and driving task data were categorised in the same manner as saliva collection (described in Section 2.3), thus each saliva sample corresponded to a performance trial.

2.4.1. PVT -B

The PVT-B is a 3 min validated (Basner et al., 2011) version of the standardised 10 min PVT (Dinges and Powell, 1985) administered on a hand-held device (PVT-192; Ambulatory Monitoring Inc., New York, USA). Participants were required to respond as quickly as possible to a millisecond counter (visual stimulus) presented on the screen of the hand-held device by pressing a button with their dominant thumb. The stimulus was presented at random intervals between 1–4 s.

Response speed on the task was investigated by assessing the reciprocal-transform of mean reaction time (mean RRT) and mean slowest 10% RT (mean slowest 10% RRT; the average of the 10% slowest reaction times during the task). The total number of lapses (i.e. RT ≥ 355 ms) were also investigated as a measure of sustained attention on the task. These measures have been identified as the ideal measures of alertness with the largest effect sizes during acute total sleep deprivation (Basner and Dinges, 2011). Data pertaining to the mitigating effects of early-morning caffeine gum administration on the total number of lapses (i.e. differences between placebo and caffeine condition at each performance trial) have been published elsewhere (Paech et al., 2016).

2.4.2. Simulated driving task

The simulated driving task has been previously described (Johnson et al., 2016). Briefly, each participant was assigned a single simulated vehicle station (configured with an automatic transmission) that consisted of a computer monitor screen, a headset, steering wheel, control pedals, and an adjustable driver's seat mounted on motion actuators so to mimic the motion of the vehicle along a sealed road (Stokes et al., 2015). The simulated driving task (Virtual Battlespace-2 software; Bohemia Interactive Simulations, Florida, USA) was designed to mimic a 40 min rural highway daytime drive along a slightly curved road with no other vehicles, intersections, or obstacles present. 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.

Driving performance measures included speed deviation (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) and lane deviation (average deviation of the geometrical centre of the vehicle from the centre of the road, excluding events where the vehicle veered off the road/crashed). The mitigating effects of caffeine gum administration on simulated driving performance (i.e. differences between placebo and caffeine condition at each performance trial) during extended wakefulness have been reported elsewhere (Johnson et al., 2016).

2.5. Statistical analyses

All data were analysed with mixed-effects models computed using Stata v.14 software (StataCorp, Texas, USA). Maximum likelihood estimation was used for all analyses (Rabe-Hesketh and Skrondal, 2012). sAA values and speed deviation measures were log-transformed to meet model assumptions (i.e. distribution and homoscedasticity). Two separate mixed-effects growth curve models (conditional and unconditional) were performed to test hypotheses. Conditional mixed-effect analyses included data pertaining to both conditions (placebo and caffeine; $n = 23$) and were used to investigate whether there were differences in circadian profiles of sAA and performance measures between the caffeine and placebo conditions. Unconditional mixed-effects analyses were restricted to data pertaining to only the caffeine condition ($n = 12$) and were performed to define diurnal profiles of sAA and performance measures during each study day in the caffeine condition, as per analysis performed on data pertaining to only the placebo condition (Pajcin et al., 2017).

For conditional mixed-effects growth curve models, fixed-effects of 'Time', 'Time^2', 'Day', 'Day × Time', and 'Day × Time^2' denote changes in dependent variables (i.e. PVT-B measures, driving performance, sAA) across time during each study day in the placebo condition. The variables 'Time' and 'Time^2' represent the linear and quadratic slopes, respectively, across Day 1. The fixed-effect of study day ('Day') indicates the change from Day 1 to Day 2 (i.e. average difference) at 09:00 h for performance or 08:00 h sAA (i.e. start of study day; intercept), and 'Day × Time' and 'Day × Time^2' represent differences in linear and quadratic slopes, respectively between study days. Results pertaining to changes in dependent variables (sAA and performance measures) in the placebo condition have been reported previously (Pajcin et al., 2017) and are only considered in

Table 2
Results of conditional growth curve mixed-effects models for measures of performance on the brief psychomotor vigilance task (PVT-B) and simulated driving task, and salivary α-amylase during two days of total sleep deprivation.

	Mean RRT		Mean Slowest 10% RRT		Total Lapses (≥ 355 ms)		Speed deviation		Lane deviation		Salivary α-amylase (sAA)	
	Coefficient (SE)	*p* value	Coefficient (SE)	*p* value	Coefficient (SE)	*p* value	Coefficient (SE)	*p* value	Coefficient (SE)	*p* value	Coefficient (SE)	*p* value
Fixed-effects												
Intercept	4.695 (0.151)	< 0.001	2.973 (0.189)	< 0.001	2.440 (1.587)	0.124	1.180 (0.126)	< 0.001	0.726 (0.081)	< 0.001	4.644 (0.252)	< 0.001
Time	0.072 (0.056)	0.198	0.174 (0.082)	0.033	−1.447 (0.713)	0.043	−0.189 (0.053)	< 0.001	−0.099 (0.036)	0.006	0.142 (0.096)	0.136
Time^2	−0.028 (0.008)	< 0.001	−0.045 (0.011)	< 0.001	0.361 (0.096)	< 0.001	0.050 (0.007)	< 0.001	0.027 (0.005)	< 0.001	−0.037 (0.012)	0.002
Day	−1.167 (0.124)	< 0.001	−1.417 (0.180)	< 0.001	11.326 (1.578)	< 0.001	1.575 (0.118)	< 0.001	1.008 (0.080)	< 0.001	−0.303 (0.189)	0.109
Day × Time	0.267 (0.079)	0.001	0.388 (0.114)	0.001	−2.210 (0.998)	0.027	−0.036 (0.075)	0.632	−0.102 (0.051)	0.046	0.106 (0.113)	0.347
Day × Time^2	−0.026 (0.011)	0.014	−0.047 (0.016)	0.003	0.201 (0.136)	0.139	−0.011 (0.010)	0.277	0.007 (0.007)	0.248	−0.014 (0.014)	0.341
Condition	0.154 (0.210)	0.462	0.305 (0.262)	0.245	−1.352 (2.198)	0.538	0.036 (0.174)	0.838	−0.014 (0.111)	0.900	0.189 (0.348)	0.588
Condition × Time	−0.113 (0.078)	0.146	−0.267 (0.113)	0.018	1.696 (0.988)	0.086	0.147 (0.074)	0.047	0.081 (0.050)	0.108	−0.185 (0.131)	0.158
Condition × Time^2	0.028 (0.010)	0.008	0.053 (0.015)	0.001	−0.354 (0.133)	0.008	−0.042 (0.010)	< 0.001	−0.023 (0.007)	0.001	0.031 (0.016)	0.054
Condition × Day	0.775 (0.172)	< 0.001	0.886 (0.250)	< 0.001	−7.451 (2.184)	0.001	−1.149 (0.163)	< 0.001	−0.744 (0.111)	< 0.001	0.189 (0.259)	0.466
Condition × Day × Time	−0.137 (0.109)	0.209	−0.093 (0.158)	0.558	0.406 (1.382)	0.769	−0.032 (0.103)	0.758	0.058 (0.070)	0.410	0.103 (0.155)	0.506
Condition × Day × Time^2	0.009 (0.015)	0.522	0.005 (0.022)	0.802	0.031 (0.188)	0.870	0.030 (0.014)	0.031	0.002 (0.010)	0.807	−0.019 (0.020)	0.333
Random-effects												
Intercept	0.157 (0.048)		0.193 (0.061)		12.372 (3.983)		0.088 (0.028)		0.031 (0.010)		0.398 (0.120)	
Residual	0.105 (0.008)		0.222 (0.017)		16.984 (1.339)		0.095 (0.007)		0.044 (0.081)		0.121 (0.010)	

Conditional growth curve mixed-effects models investigated differences in diurnal profiles of PVT-B, simulated driving, and sAA measures between the caffeine and placebo conditions. Time and Time^2 represent linear and quadratic slopes of dependent variables over time. The time variable was centred at 08:00 h for sAA measures and 09:00 h for PVT-B and simulated driving measures, and increases by one every 3 h of wakefulness per study day. The study day variable was centred at Day 1 and represents the difference between study days at the intercept. Condition represents the difference between the caffeine and placebo conditions at the intercept, while its interactions with time and day denote changes in slopes during each study in the caffeine condition as compared with the placebo condition. Analyses were performed on log-transformed sAA measures and speed deviation values. Reciprocal-transform of mean reaction time (mean RRT) and mean slowest 10% reaction times (mean slowest 10% RRT) were analysed. Mean RRT, mean slowest 10% RRT, and total lapses represent outcomes of PVT-B. Speed deviation and lane deviation are measures of performance during simulated driving task.

the present article for comparison with the the caffeine condition. Fixed-effects of 'Condition' and its interaction with time ('Condition × Time'; 'Condition × Time^2') and study day ('Condition × Day'; 'Condition × Day × Time'; 'Condition × Day × Time^2') were included in conditional mixed-effects models to investigate differences in profiles of dependent variables between the placebo and caffeine conditions. The fixed-effect of 'Condition' represents differences between the placebo and caffeine condition at the intercept (i.e. 09:00 h for performance and 08:00 h for sAA), while 'Condition × Time' and 'Condition × Time^2' interactions represent differences in linear and quadratic slopes between the placebo and caffeine conditions during Day 1. The fixed-effects 'Condition × Day', 'Condition × Day × Time' and 'Condition × Day × Time^2' denote differences at intercept, and linear and quadratic slopes, respectively between study days (i.e. change from Day 1 to Day 2) between the placebo and caffeine conditions.

For all models, the time variable was centred at 08:00 h for sAA and 09:00 h for performance measures (i.e. commencement of saliva collection and performance assessment, respectively), and increased by one unit every three hours of wakefulness. Thus, the intercept represents the average of the dependent variable at 08:00 h or 09:00 h, the linear slope (Time) represents the average instantaneous change from 08:00 h or 09:00 h, and the quadratic slope (Time^2) describes the degree and direction of curvature in the dependent variable across the day. It should be noted, that saliva was not collected at 08:00 h on Day 1 and the measures presented are marginal estimates (i.e. predicted values) derived from the quadratic growth curve mixed-effects models. A random-effects intercept was included to account for between-subject variability and the residual estimate represented the within-subject variability. Residuals were checked for normality and heteroscedasticity. All estimates of model parameters are reported as the coefficient (β) ± standard error (SE).

To investigate whether the relationship between sAA and performance on the PVT-B and simulated driving task previously identified in the placebo condition (Pajcin et al., 2017) persisted in the caffeine condition, separate linear mixed-effects models were performed with fixed-effects of log-transformed sAA measures and performance measures as dependent variables. Results reported are coefficient (β) ± SE, with the coefficient indicating the average change in the dependent variable (performance) with every unit increase in sAA. Effect sizes were calculated using Cohen's f^2 effect size measure (Selya et al., 2012). Only data pertaining to the caffeine condition were included in these separate mixed-effects analyses ($n = 12$). Data pertaining to the association between sAA and neurobehavioural performance in the placebo condition are reported elsewhere (Pajcin et al., 2017).

Significance was assumed at $p \leq 0.05$. All data reported are mean ± SE, unless indicated otherwise. All graphs are estimated marginal means derived from the conditional quadratic growth curve mixed-effects models ± SE, unless indicated otherwise.

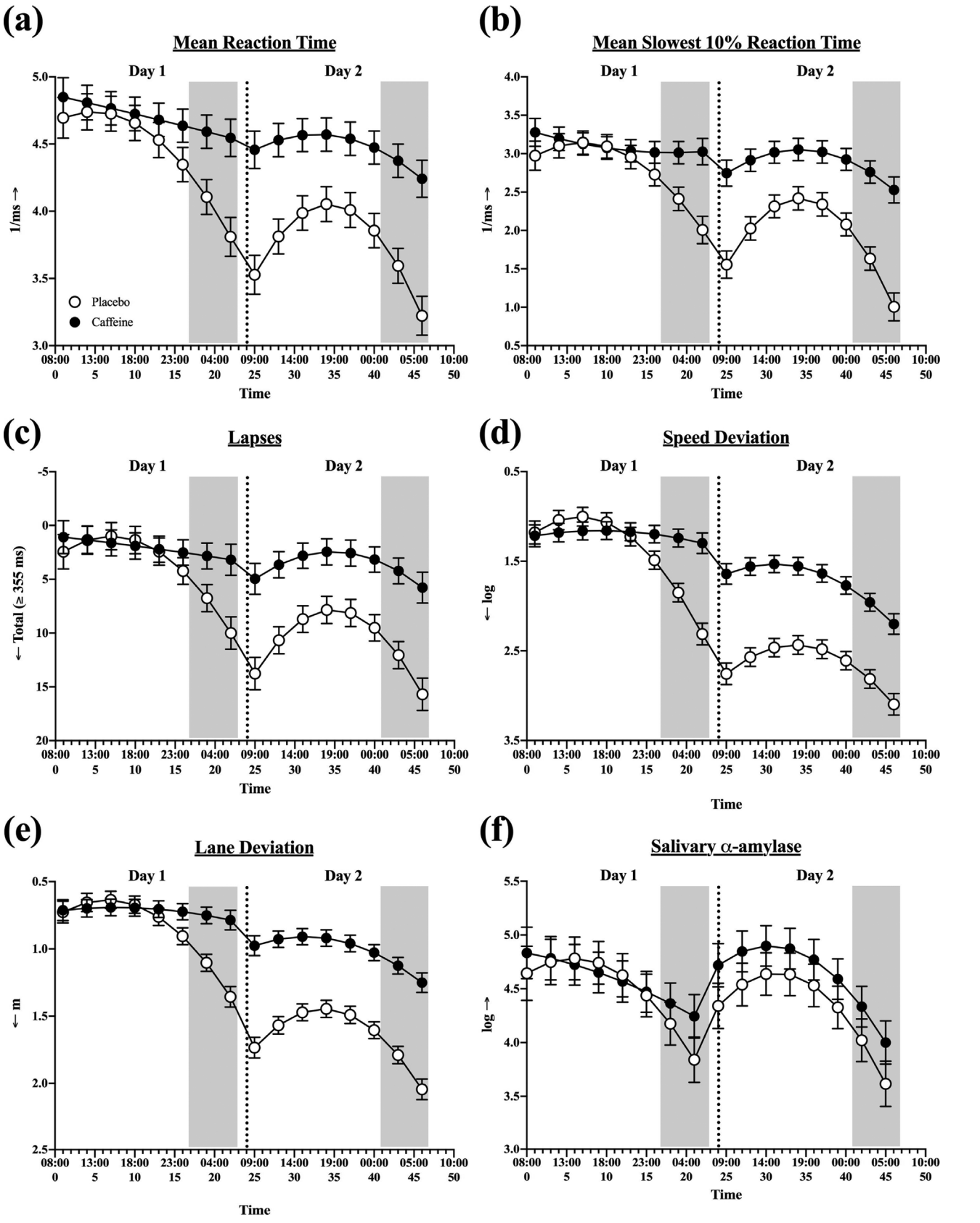
3. Results

Conditional quadratic growth curve mixed-effects models were performed to determine whether the profiles of sAA and neurobehavioural performance in the caffeine condition differed from the previously reported findings in the placebo condition (Pajcin et al., 2017). Unconditional quadratic growth curve mixed-effects models were restricted to data pertaining only to the caffeine condition and performed to establish the diurnal profiles of sAA and neurobehavioural performance measures in the caffeine condition, as per previous analysis in the placebo condition (Pajcin et al., 2017).

3.1. PVT-B

Results of conditional mixed-effects models for PVT-B measures (mean RRT, mean slowest 10% RRT, and lapses) are presented in Table 2. The significant interaction between condition and time

(Condition × Time^2) and non-significant three-way interactions of condition with time and day (Condition × Day × Time; Condition × Day × Time^2) revealed that the temporal profiles of PVT-B measures exhibited in the caffeine condition were significantly different to the placebo condition during Day 1, but not Day 2 (Table 2; Fig. 2a–c).



(caption on next page)

Fig. 2. Measures of performance on brief psychomotor vigilance test (PVT-B) and simulated driving task, and salivary α-amylase levels in the caffeine (●) and placebo (○) conditions during 50 h (i.e. two days) of total sleep deprivation.
Caffeine gum attenuated the circadian decline in reciprocal-transform of mean reaction time (mean RRT) (a) and mean slowest 10% reaction time (mean slowest 10% RRT) (b), total number of lapses (c), speed deviation (d), and lane deviation (e) during Day 1 in the caffeine condition, as compared with the placebo condition. There was also a near-significant ($p = 0.054$) attenuation of the circadian decline in salivary α-amylase (sAA) (f) during Day 1 in the caffeine condition, as compared with the placebo condition. During Day 2, performance on the PVT-B (mean RRT (a), mean slowest 10% RRT (b), lapses (c)) and simulated driving task (speed deviation (d), lane deviation(e)), and salivary α-amylase measures (f) in the caffeine condition exhibited circadian profiles that were not significantly different to the placebo condition. Reciprocal-transform of mean reaction time (a) and mean slowest 10% reaction time (b) are presented. Log-transformed values of speed deviation (d) and salivary α-amylase (f) are presented. The Y-axes of lapses (c), speed deviation (d), and lane deviation (e) are reversed to facilitate interpretation, thus downwards on the Y-axes indicates impaired performance. White circles represent placebo condition and black circles represent caffeine condition. Data are estimated marginal means ± SE derived from conditional growth curve mixed-effects models. Dashed line at 08:00h denotes categorisation of data into Day 1 (0–24 h wakefulness) and Day 2 (24–48 h wakefulness). Timing of caffeine gum administration is represented by grey columns on Day 1 and Day 2. Upper x-axes represent 24 h clock time (hh:mm) and lower x-axes represent the total hours of wakefulness.

At the beginning of the study, there were no significant differences in mean RRT, mean slowest 10% RRT, and lapses between conditions, as indicated by the non-significant 'Condition' variable (Table 2). There were also no significant differences between conditions in the linear slopes of mean RRT and lapses during Day 1 (Condition × Time; Table 2), indicating these measures were stable during the beginning of the day in the caffeine condition, consistent with performance in the placebo condition (Fig. 2a and c). In contrast, there was a significant decrease in the linear slope of mean slowest 10% RRT (Condition × Time, $p < 0.05$; Table 2) in the caffeine condition as compared with the placebo (Fig. 2b), suggesting mean slowest 10% RRT was reduced (i.e. performance enhanced) in the caffeine condition as compared with the placebo condition during Day 1 (Fig. 2b).

The significant condition and quadratic time interaction variable (Condition × Time^2, $p < 0.01$; Table 2) indicated there was a difference in the curvature of PVT-B measures during Day 1 in the caffeine condition, as compared with the placebo condition (Fig. 2a–c). Results showed the circadian decline in PVT-B performance was attenuated during the evening and early-morning of Day 1 in the caffeine condition (Fig. 2a–c). Unconditional mixed-effects growth curve models (Table 3) revealed that, unlike the placebo condition, performance on the PVT-B in the caffeine condition was stable and unchanged during Day 1. Non-significant linear and quadratic (Time and Time^2; Table 3) slopes indicated there was no change in mean RRT (Fig. 2a), mean slowest 10% RRT (Fig. 2b), or lapses (Fig. 2c) during Day 1 in the caffeine condition.

As wakefulness continued into Day 2, the change in response speed (mean RRT and mean slowest 10% RRT) and lapses on the PVT-B between study days was significantly different between conditions. The interaction between condition and study day (Condition × Day, $p < 0.001$; Table 2) revealed that response speed and number of lapses in the placebo condition, were significantly increased and decreased, respectively in the caffeine condition. Mean RRT and mean slowest 10% RRT data are reciprocal-transform, thus an increase is indicative of enhanced performance. However, the non-significant three-way interactions (Condition × Day × Time and Condition × Day × Time^2; Table 2) indicated there were no differences in linear and quadratic slopes between conditions during Day 2, suggesting that PVT-B performance in the caffeine condition exhibited circadian profiles similar to the placebo condition during Day 2 (Fig. 2a–c). Results of unconditional mixed-effects growth curve models (Table 3) revealed there was a significant decline in response speed (mean RRT and mean slowest 10% RRT) and increase in the number of lapses on Day 2 in the caffeine condition (Day, $p < 0.001$; Table 3). Thus, despite PVT-B performance being enhanced in the caffeine condition as compared with placebo (Fig. 2a–c), performance measures in the caffeine condition exhibited circadian profiles (Fig. 2a–c). Significant linear slope and study day interactions (Day × Time, $p < 0.05$; Table 3) indicated there was a small increase in mean RRT and mean slowest 10% RRT and decrease in total number of lapses in the caffeine condition, suggesting a small improvement in performance on the PVT-B during the waking-day of Day 2 (Fig. 2a–c). This was followed by a steady decline as wakefulness increased into the evening and early-morning (Fig. 2a–c) (Day × Time^2; Table 3).

3.2. Simulated driving task

Similar to performance on the PVT-B, condition and time (Condition × Time; Condition × Time^2) and condition with time and study day interactions (Condition × Day × Time; Condition × Day × Time^2) of conditional mixed-effects models (Table 2) indicated there were differences in circadian profiles of driving performance measures (lane deviation and speed deviation) between the caffeine and placebo conditions during Day 1, but not Day 2.

There were no significant differences in driving performance between conditions at the beginning of the study (Condition; Table 2). Non-significant condition by time interaction (Condition × Time; Table 2) for lane deviation indicated there was no difference in linear slope between conditions during Day 1 (Fig. 2e). However, there was a significant difference in the linear slope for speed deviation between conditions (Condition × Time, $p < 0.05$; Table 2). The results indicated that the decline observed in the placebo condition (Time, $p < 0.001$; Table 2) was attenuated in the caffeine condition (Fig. 2d). The significant condition and quadratic time interaction (Condition × Time^2, $p < 0.001$; Table 2) indicated there was a difference between conditions in the curvature of driving performance measures during Day 1 (Fig. 2d and e). The negative coefficients of the Condition X Time^2 interaction indicated that the increase in speed and lane variability that was observed in the placebo condition, was attenuated in the caffeine condition (Table 2; Fig. 2d and e). An increase in speed and lane variability is indicative of impaired driving performance thus, an attenuation indicates improved performance. Results of unconditional mixed-effects models (Table 3) revealed there were no changes in speed deviation and lane deviation during Day 1 (Time and Time^2; Table 3), confirming caffeine administration attenuated the circadian decline in driving performance (Fig. 2d and e). It should be noted that Y-axes in Fig. 2d and e have been revered to facilitate interpretation.

During Day 2, driving performance was significantly enhanced in the caffeine condition as compared with the placebo condition (Fig. 2d and e) (Condition × Day, $p < 0.001$; Table 2). However, non-significant condition by day and time interactions (Condition × Day × Time and Condition × Day × Time^2; Table 2) revealed there were no differences in linear and quadratic slopes of lane deviation between conditions during Day 2, suggesting that the circadian profile of lane deviation exhibited by the caffeine condition was similar to the placebo condition (Fig. 2e). There was no significant difference in linear slope of speed variability in the caffeine condition as compared with the placebo condition during Day 2 (Condition × Day × Time; Table 2). However, the significant condition by study day and quadratic slope interaction (Condition × Day × Time^2, $p < 0.05$; Table 2) suggested the increase in speed deviation in the placebo condition was significantly less pronounced in the caffeine condition (Fig. 2d). Separate unconditional mixed-effect models (Table 3) revealed there were no changes in linear slopes of speed deviation or lane deviation during Day 2 as compared with Day 1 in the caffeine condition (Day × Time; Table 3). However, the interaction between day and quadratic slope for time of day (Day × Time^2, $p < 0.05$; Table 3) indicated speed deviation steadily increased with increasing time awake across Day 2 (Fig. 2d) and there was a trend towards an increase in lane deviation

Table 3
Results of unconditional growth curve mixed-effects models for measures of performance on the brief psychomotor vigilance task (PVT-B) and simulated driving task, and salivary α-amylase during two days of total sleep deprivation.

	Mean RRT		Mean Slowest 10% RRT		Total Lapses (> 355 ms)		Speed deviation		Lane deviation		Salivary α-amylase (sAA)	
	Coefficient (SE)	*p* value	Coefficient (SE)	*p* value	Coefficient (SE)	*p* value	Coefficient (SE)	*p* value	Coefficient (SE)	*p* value	Coefficient (SE)	*p* value
Fixed-effects												
Intercept	4.849 (0.133)	< 0.001	3.278 (0.147)	< 0.001	1.087 (0.943)	0.249	1.216 (0.132)	< 0.001	0.712 (0.080)	< 0.001	4.833 (0.208)	< 0.001
Time	−0.041 (0.046)	0.376	−0.093 (0.065)	0.155	0.250 (0.456)	0.584	−0.042 (0.051)	0.407	−0.019 (0.029)	0.526	−0.042 (0.097)	0.662
Time^2	−0.0004 (0.006)	0.950	0.008 (0.009)	0.358	0.007 (0.062)	0.909	0.008 (0.007)	0.260	0.004 (0.004)	0.292	−0.006 (0.012)	0.615
Day	−0.392 (0.101)	< 0.001	−0.532 (0.144)	< 0.001	3.874 (1.008)	< 0.001	0.426 (0.112)	< 0.001	0.264 (0.065)	< 0.001	−0.114 (0.192)	0.554
Day × Time	0.130 (0.064)	0.043	0.295 (0.091)	0.001	−1.804 (0.638)	0.005	−0.068 (0.071)	0.340	−0.044 (0.041)	0.288	0.209 (0.115)	0.068
Day × Time^2	−0.017 (0.009)	0.055	−0.041 (0.012)	0.001	0.232 (0.087)	0.008	0.019 (0.010)	0.045	0.010 (0.006)	0.064	−0.033 (0.015)	0.025
Random-effects												
Intercept	0.143 (0.061)		0.120 (0.053)		3.837 (1.773)		0.124 (0.053)		0.048 (0.020)		0.176 (0.076)	
Residual	0.077 (0.008)		0.155 (0.017)		7.563 (0.825)		0.093 (0.010)		0.031 (0.003)		0.142 (0.015)	

Unconditional growth curve mixed-effects models were performed to determine the profiles of PVT-B and simulated driving task performance measures, and salivary α-amylase (sAA) levels in the caffeine condition. Time and Time^2 represent linear and quadratic slopes of dependent variables over time and were centred at 08:00 h for sAA measures and 09:00 h for performance, and increase by one every 3 h of wakefulness per study day. The study day variable was centred at Day 1 and represents the difference between study days at the intercept. Analyses were performed on log-transformed sAA measures and speed deviation values. Reciprocal-transform of mean reaction time (mean RRT) and mean slowest 10% reaction times (mean slowest 10% RRT) were analysed. Mean RRT, mean slowest 10% RRT, and total lapses represent outcomes of PVT-B. Speed deviation and lane deviation are measures of performance during simulated driving task.

(Day × Time^2, $p = 0.064$) (Fig. 2e). Despite driving performance being enhanced in the caffeine condition, relative to the placebo condition (Fig. 2d and e), speed deviation and lane deviation were significantly increased on Day 2 in the caffeine condition as compared with Day 1 (Day, $p < 0.001$; Table 3).

3.3. Salivary α-amylase

Conditional mixed-effects analysis revealed there was a trend towards a difference between the caffeine and placebo conditions in the circadian profile of sAA (Table 2). Similar to performance measures, the near-significant interaction between condition and quadratic slope for time of day (Condition × Time^2, $p = 0.054$; Table 2) suggested there was a trend towards a difference between conditions in the curvature of sAA during Day 1 (Fig. 2f). The positive coefficient of the Condition X Time^2 interaction indicated there was a trend towards an attenuation of the diurnal decline in sAA during Day 1 in the caffeine condition (Fig. 2f). As was the case with performance tasks, results of unconditional mixed-effects analysis (Table 3) indicated that sAA levels were stable and unchanged during Day 1 in the caffeine condition (Fig. 2f). These findings are in contrast to the circadian decline observed in the placebo condition (Fig. 2f).

As wakefulness continued into Day 2, unlike performance measures, sAA levels were not significantly increased in the caffeine condition as compared with the placebo condition (Condition × Day; Table 2). However, the non-significant condition by study day and time of day interactions (Condition × Day × Time and Condition × Day × Time^2; Table 2) indicated there were no differences in linear and quadratic slopes of sAA during Day 2 between conditions, suggesting that sAA in the caffeine condition exhibited a circadian profile similar to the placebo condition during Day 2 (Fig. 2f).

Results of unconditional mixed-effects models (Table 3) revealed there was no difference in sAA between study days in the caffeine condition (Day; Table 3), but sAA did exhibit a circadian profile during Day 2 (Fig. 2f). There was trend towards an increase sAA during the waking-day of Day 2 (Day × Time, $p = 0.064$; Table 3) (Fig. 2f), followed by a steady decline (Day × Time^2, $p < 0.05$; Table 3) as wakefulness increased into the evening and early-morning of Day 2 (Fig. 2f). These findings are consistent with diurnal profile observed in the placebo condition (Fig. 2f), and suggest that caffeine did not attenuate the diurnal decline in sAA during Day 2.

3.3.1. Association with PVT-B

Log-transformed sAA values were positively associated with mean RRT (0.134 ± 0.052, $p = 0.011$; $f^2 = 0.06$) (Fig. 3a) and negatively associated with total number of lapses (−0.991 ± 0.493, $p = 0.044$; $f^2 = 0.01$) (Fig. 3c) in the caffeine condition across the two days of total sleep deprivation. There was no significant association between log-transformed sAA values and mean slowest 10% RRT (0.115 ± 0.072, $p = 0.109$) (Fig. 3b). When mixed-effects models were restricted to only data pertaining to Day 1, the positive association between sAA and mean RRT remained (0.182 ± 0.063, $p = 0.004$; $f^2 = 0.14$) (Fig. 3a) and a significant positive association between sAA and mean slowest 10% RRT was observed (0.204 ± 0.083, $p = 0.013$; $f^2 = 0.09$) (Fig. 3b). The significant negative association between sAA values and total number of lapses remained (−1.634 ± 0.502, $p = 0.001$; $f^2 = 0.14$) (Fig. 3c). The significant positive association between sAA values and mean RRT (0.152 ± 0.075, $p = 0.043$; $f^2 = 0.05$) also remained when mixed-effects models were restricted to data pertaining to only Day 2 (Fig. 3a), but the associations with mean slowest 10% RRT (0.133 ± 0.107, $p = 0.210$) (Fig. 3b) and total number of lapses (−1.117 ± 0.737, $p = 0.130$) were not significant (Fig. 3c).

3.3.2. Association with simulated driving task

There was no association between sAA and speed deviation (−0.066 ± 0.074, $p = 0.368$) or lane deviation (−0.020 ± 0.041,

$p = 0.621$) in the caffeine condition across the 50 h of sleep deprivation (Fig. 4). There was also no significant association between sAA and driving performance measures when mixed-effects models were restricted to Day 1 (speed deviation: −0.093 ± 0.070, $p = 0.187$; lane deviation: −0.008 ± 0.034, $p = 0.808$) (Fig. 4). When mixed-effects models were restricted to Day 2, a significant negative association between sAA values and speed deviation (-0.258 ± 0.080, $p = 0.001$; $f^2 = 0.15$) (Fig. 4a) and lane deviation (−0.109 ± 0.047, $p = 0.021$; $f^2 = 0.10$) (Fig. 4b) was observed in the caffeine condition.

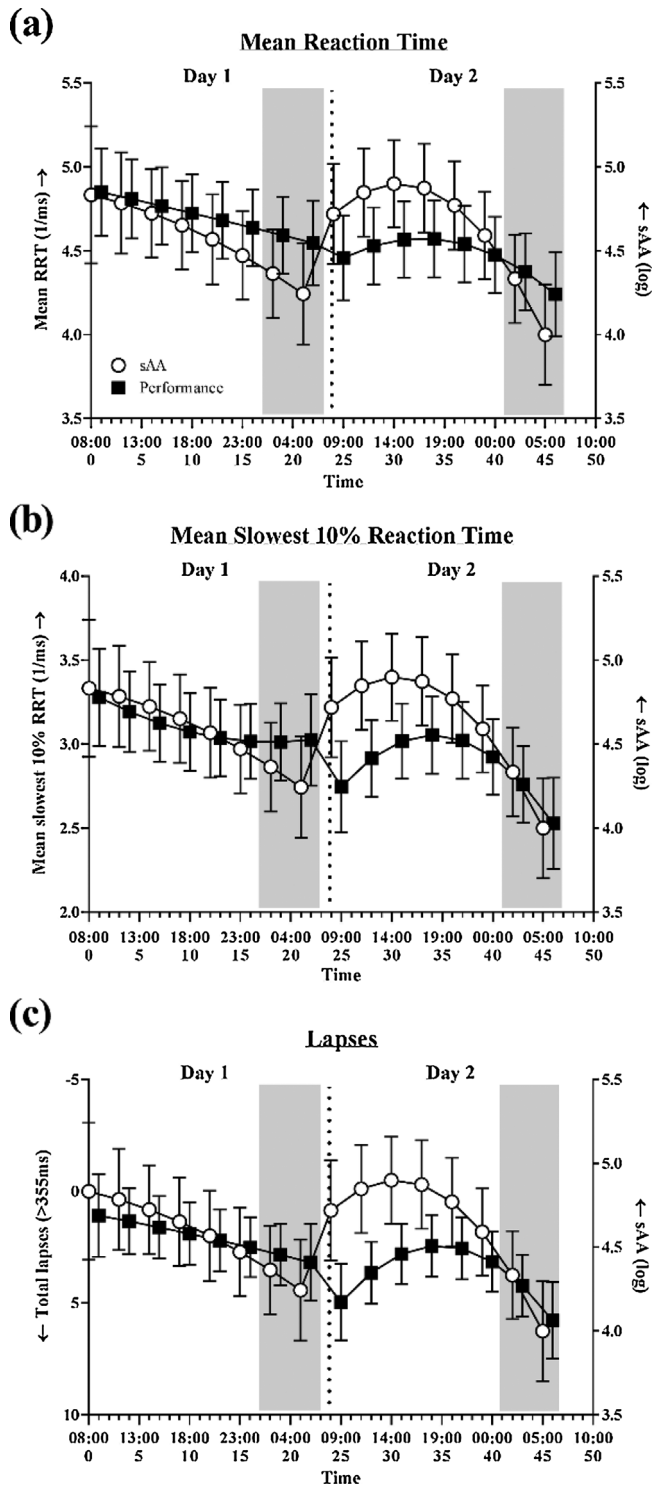


Fig. 3. Marginal estimates derived from mixed-effects models of performance measures on the brief psychomotor vigilance test (PVT-B) (■) superimposed with log-transformed salivary α-amylase (sAA) measures (○) across two days (i.e. 50 h) of total sleep deprivation in the caffeine condition.
There was a significant association between salivary α-amylase (sAA) and reciprocal-transform of mean reaction time (mean RRT) (a) and total number of lapses (c) across the two days of total sleep deprivation. When analyses were restricted to Day 1, sAA levels were associated with mean RRT (a), mean slowest 10% RRT (b), and total number of lapses (c), suggesting an increase in sAA was associated with improved response speed (mean RRT and mean slowest 10% RRT) and sustained attention (lapses) on the PVT-B. During Day 2, the significant association between sAA and mean RRT (a) remained, but there was no association with mean slowest 10% RRT (b) and total number of lapses (c). Reciprocal-transform of mean reaction (a) and mean slowest 10% reaction time (b) are presented. Estimated marginal means are derived from unconditional mixed-effects models (i.e. caffeine condition only). White circles represent log-transformed sAA measures and black squares represent PVT-B measures. Data are mean ± 95% CI. PVT-B measures are presented on left Y-axes and log-transformed sAA measures are presented on the right Y-axes. Left Y-axis of lapses (c) has been reversed to facilitate interpretation (i.e. decline on graph indicates impaired performance). Timing of caffeine gum administration is represented by grey columns on Day 1 and Day 2. Upper X-axes represent 24 h clock time (hh:mm) and lower X-axes represent the total hours of swakefulness.

4. Discussion

The main objective of this article was to investigate whether the previously reported association between sAA and neurobehavioural performance during two days of total sleep deprivation in the placebo condition (Pajcin et al., 2017) persisted in the presence of caffeine as a sleepiness countermeasure. Results show that the effects of strategic early-morning caffeine gum administration on the circadian profile of sAA across two days of sleep deprivation coincided with the variations in neurobehavioural performance measures. However, unlike performance, sAA was not significantly increased in the caffeine condition. Further, the association between higher sAA and enhanced performance, as per our previous findings in the placebo condition (Pajcin et al., 2017), was only evident with mean RRT consistently in the caffeine condition across both days of sleep deprivation. The associations between sAA and other measures of performance in the caffeine condition were not consistent across both study days.

Waking performance is mediated by the interaction between the circadian propensity for alertness and the homeostatic pressure for sleep (Dijk et al., 1992; Achermann and Borbely, 1994). Thus, a suitable physiological measure of operator performance must be sensitive to both processes in the same manner. In the current study, results show that early-morning caffeine gum administration successfully attenuated the circadian decline in performance on the PVT-B and simulated driving task during Day 1, but not Day 2. Performance in the caffeine condition was stable and unchanged during Day 1, which is in contrast to the steady decline reported in the placebo condition (Pajcin et al., 2017). However, as wakefulness continued into Day 2, the circadian profile exhibited by performance was not significantly different to the placebo condition. Consistent with the placebo condition (Pajcin et al., 2017), performance in the caffeine condition declined during the evening and early-morning of Day 2, suggesting that caffeine gum on Day 2 did not effectively attenuate the impact of the increased sleep propensity on performance. These caffeine-mediated changes in the circadian profiles of performance were consistent with variations in sAA. Similar to neurobehavioural performance, there was a near-significant attenuation of the circadian decline in sAA in the caffeine condition during Day 1, and as wakefulness continued in Day 2, sAA measures exhibited a circadian profile that was not significantly different to the placebo condition. These findings suggest that caffeine-mediated variations in sAA secretion may be regulated by similar mechanisms that modulate changes in neurobehavioural performance.

During extended wakefulness, caffeine enhances alertness and performance in a dose-dependent manner (Ramakrishnan et al., 2014) by overriding the effects of increased sleep pressure (Urry and Landolt, 2015; McLellan et al., 2016). In the present study, the attenuation of the

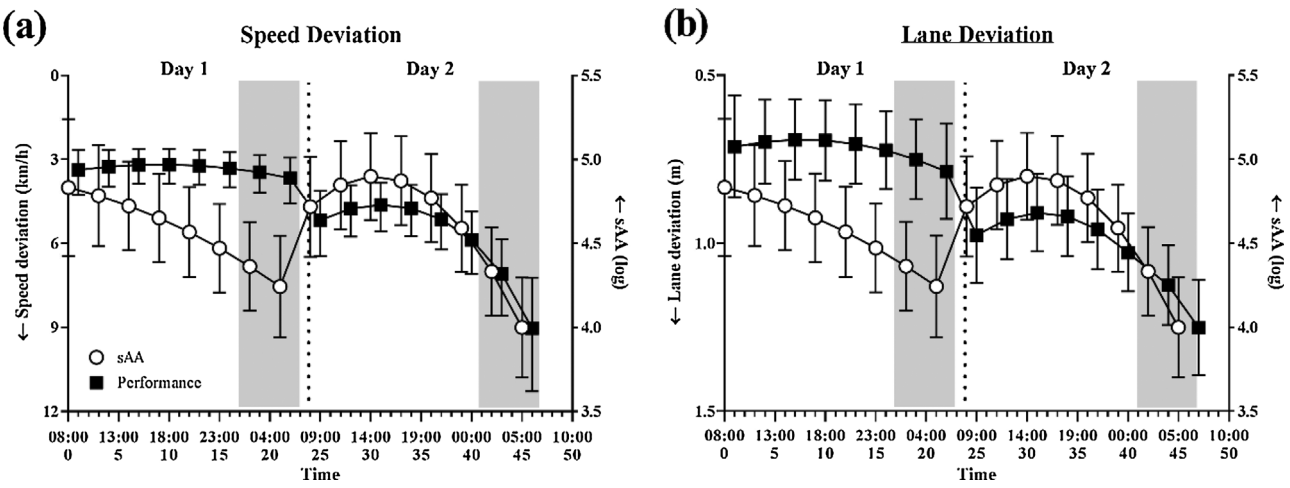


Fig. 4. Marginal estimates derived from mixed-effect models of driving performance measures (■) superimposed with log-transformed salivary α-amylase (sAA) measures (○) across two days (i.e. 50 h) of total sleep deprivation in the caffeine condition.

There was no significant association between sAA and simulated driving performance during Day 1. There was a significant negative association between sAA and speed deviation (a) and lane deviation (d) during Day 2, suggesting elevated sAA was associated with improved driving performance only during 24–48 h of sustained wakefulness. Analyses were performed on log-transformed values of speed deviation, but anti-logged values are presented to facilitate interpretation (a). Estimated marginal means are derived from unconditional mixed-effects models (i.e. caffeine condition only). White circles represent log-transformed sAA measures and black squares represent simulated driving task outcomes. Data are mean ± 95% CI. Driving performance measures are presented on left Y-axes and log-transformed sAA measures are presented on the right Y-axes. Left Y-axes of speed deviation (a) and lane deviation (b) have been reversed to facilitate interpretation (i.e. decline on graph indicates impaired performance). Timing of caffeine gum administration is represented by grey columns on Day 1 and Day 2. Upper X-axes represent 24 h clock time (hh:mm) and lower X-axes represent the total hours of wakefulness.

circadian decline in sAA and performance in the caffeine condition on Day 1, may be explained by the sympathomimetic properties of caffeine (Benowitz et al., 1995; Arciero et al., 1998; Fitzgerald, 2013). Blockade of adenosine signalling by caffeine (Fredholm, 1995) results in decreased inhibitory adenosine modulation and consequent enhanced NA release (Fisone et al., 2004). Increased NA secretion stimulates SNS activation (Esler et al., 1985), which is responsible for the classical symptoms of increased heart rate, blood pressure and temperature associated with caffeine intake (Bunsawat et al., 2015; Zimmermann-Viehoff et al., 2016). Elevated central and peripheral levels of NA have been associated with improved vigilance and alertness (Smith and Nutt, 1996; Bellesi et al., 2016), and caffeine-mediated increased arousal has been linked with improvement in neurocognitive functioning during sleep loss (Wyatt et al., 2004). Similarly, numerous studies have documented the significant role of NA in sAA secretion (Nater and Rohleder, 2009), and higher sAA levels have been associated with improved vigilance (Muehlhan et al., 2013; Pajcin et al., 2017). However, with continuous sleep loss the homeostatic pressure for sleep eventually surpasses the arousal-promoting effects of caffeine, and performance begins to decline (Johnson et al., 2016; Paech et al., 2016). It is plausible that in the present study, as wakefulness continued into Day 2 the homeostatic pressure for sleep exceeded the stimulatory effects of caffeine, and the second regime of caffeine was not effective at attenuating the circadian decline mediated by the increased propensity for sleep. Thus, neurobehavioural performance and sAA measures in the caffeine condition exhibited circadian profiles during Day 2 that were not different to the placebo condition.

Although there were no differences in circadian profiles of performance and sAA between the caffeine and placebo conditions during Day 2, neurobehavioural performance was significantly enhanced on Day 2 in the caffeine condition as compared with the placebo. Contrary to our expectations, sAA was not significantly increased in the caffeine condition. This contradicts previous findings indicating caffeine consumption increased sAA (Bishop et al., 2006; Klein et al., 2010) and is consistent with reports that basal sAA activity is not affected by caffeine in habitual users (Nater et al., 2007; Klein et al., 2014), as all participants in the caffeine condition in the current study, except one, were habitual caffeine users (≥1 cup of coffee/day). The lack of a significant caffeine-mediated increase in sAA indicates a robust circadian rhythm. Interestingly, the circadian rhythm of sAA is similar to the rhythm of core body temperature (Wright et al., 2002) and an inverse of the endogenous rhythm of melatonin, a prominent marker of circadian phase (Lewy et al., 1999). These findings suggest that sAA may be useful as a potential peripheral surrogate circadian marker. However, further research is needed to assess the effects of caffeine consumption on circadian timing (Burke et al., 2015; St Hilaire and Lockley, 2015) and investigate sAA secretion alongside well-established circadian markers, such as melatonin (Lewy et al., 1999).

As was the case with previous work (Pajcin et al., 2017), the association between sAA and neurobehavioural performance in the caffeine condition was investigated due to similarities in circadian profiles. However, contrary to expectations and previous findings in the placebo condition (Pajcin et al., 2017), the association between sAA and neurobehavioural performance was only evident with mean RRT consistently across the entire duration of sleep deprivation. The effect size of the association between sAA and mean RRT in the caffeine condition ($f^2 = 0.06$) was markedly smaller than that reported in the placebo condition ($f^2 = 0.21$), indicating that the association in the caffeine condition was relatively weaker. There was a significant association between sAA and all PVT-B metrics during Day 1 (i.e. 0–24 h of sustained wakefulness), but no association with driving performance. During Day 2 (i.e. 24–48 h of sustained wakefulness), sAA measures were associated with driving performance and only mean RRT on the PVT-B. Associations between sAA and neurobehavioural performance were not reported separated by study day in previous work in the placebo condition (Pajcin et al., 2017). However, retrospective analysis revealed that the effects sizes of the associations between sAA and PVT-B measures in the placebo condition during Day 1 (range: 0.10–0.14) were consistent with those reported in the caffeine condition in the present article (range: 0.09–0.14). When analyses were restricted to Day 2, the effect sizes of associations between sAA and performance in the caffeine condition (range: 0.05–0.15) were markedly smaller than the placebo condition (range: 0.23–0.31). These results suggest that the association between sAA and performance was weaker in the caffeine condition on Day 2 as compared with the placebo condition.

The lack of an association between driving performance and sAA during Day 1 is unclear. It may be that the pronounced stimulatory effects of caffeine stabilised speed and lane variability across Day 1,

resulting in no variation across the day. This is inconsistent with the small variation in sAA and performance on the PVT-B, suggesting that caffeine may have had a greater impact on modulators of driving performance. The significant association between sAA and mean RRT, speed variability and lane variability during Day 2, is most likely explained by similarities in the circadian profiles and consistent with our previous proposal of sAA monitoring performance by closely tracking the circadian propensity for alertness (Pajcin et al., 2017). The lack of an association between sAA and mean slowest 10% RRT or lapses during Day 2, despite similarities in circadian profiles, may be explained by the impact of the homeostatic pressure for sleep on these PVT-B measures in comparison to sAA. Mean slowest 10% RRT and lapses are metrics of slowed response times during the PVT-B thus, these measures may show a greater impairment during extended wakefulness in comparison with mean RRT, which is a measure of the average response speed. Results show that the degree of decline in mean slowest 10% RRT and increase in lapses during Day 2 was relatively greater than the decline in mean RRT. The lack of an association with sAA may be due to there being only a small, and not significant, decline in sAA levels on Day 2 as compared with Day 1. However, sAA measures at 08:00 h on Day 1 are predicted values thus, it may be that a difference between study days was not observed in sAA because a raw sample was not collected at baseline (08:00 h on Day 1) for true comparison to the sample during extended wakefulness (i.e. 08:00 h on Day 2).

Nevertheless, the significant association between sAA and all PVT-B measures during Day 1 is consistent with previous findings in the placebo condition (Pajcin et al., 2017). Given the popularity of caffeine as a countermeasure for sleepiness-induced performance impairments during sleep loss (McLellan et al., 2016), these findings increase usefulness of sAA as a potential non-invasive physiological measure of operator performance during the first 24 h sustained wakefulness (i.e. one night of sleep deprivation) in the presence of common sleepiness-countermeasures. Studies have reported effects of other psychostimulatory substances such as modafinil and dextroamphetamine on performance and alertness comparable to caffeine during sleep deprivation (Wesensten et al., 2002; Killgore et al., 2008), suggesting results in the present study may extend to other sleepiness-countermeasures.

Findings need to be interpreted with caution as this study was carried out in a controlled laboratory setting with healthy young adults, limiting translation of results to operational environments. Caffeine administration was also strictly regulated, which is not consistent with caffeine intake in the real-world, particularly during night work (Akerstedt and Landstrom, 1998). It would be of interest to investigate whether higher doses of caffeine significantly attenuate the circadian decline in sAA during the early-morning, and the effects of other psychostimulants (e.g. modafinil) on the circadian profile of sAA. Also, exploring the effects of caffeine on sAA and performance in habitual and non-habitual consumers would be of interest as the duration of abstinence in the present study (i.e. seven days) may have resulted in an enhanced effect of caffeine on performance (Yeomans et al., 2002; Addicott and Laurienti, 2009). Further, investigation of sAA changes and association with performance in individuals who frequently engage in work for extended periods without sleep, such as emergency services (Barger et al., 2005) or the military (Lieberman et al., 2005), and during different protocols of sleep loss (e.g. chronic sleep restriction) would increase generalisability of findings to real-world. Evidence of individual differences to the effects of caffeine (Attwood et al., 2007) and sleep deprivation (Van Dongen et al., 2004; Rupp et al., 2012; Goel et al., 2015) on performance and subjective feelings of sleepiness, demonstrate the need for objective, physiological monitoring.

The current study shows that caffeine-mediated changes in the circadian profile of sAA were similar to variations in neurobehavioural performance during two days of total sleep deprivation. Contrary to previous findings in the placebo condition (Pajcin et al., 2017), the association between sAA and performance was not consistent across both days of sleep deprivation. A significant association between sAA and all PVT-B metrics in the caffeine condition was observed during the first 24 h of sustained wakefulness. The association between sAA and response speed on the PVT-B was also evident when wakefulness continued beyond 24 h and up to 48 h. The consistent association between sAA and mean RRT during sleep deprivation in the caffeine condition increases the usefulness of sAA as a potential non-invasive physiological measure of response speed. However, the lack of a consistent association between sAA and other PVT-B measures and driving performance warrants further investigation. Future studies could use a constant routine to elucidate the endogenous circadian component of sAA secretion or forced desynchrony to validate the homeostatic and circadian influences. Validation of the endogenous processes regulating the circadian rhythm of sAA secretion would increase the usefulness of sAA in research and operational environments.

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Conflict of interest

None.

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