scientific reports

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Dose response effects of theacrine on cognitive performance and subsequent sleep

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Psychostimulants can be employed as a countermeasure to cognitive declines resulting from insufficient sleep. Although caffeine is the most consumed psychostimulant, consumption can cause adverse side-effects, including sleep disturbance. Therefore, there is interest in identifying alternative supplements that improve cognitive performance without compromising subsequent sleep. Here we investigate the influence of the dose and timing of theacrine consumption on cognitive performance and subsequent sleep using conditions that replicate a low (100 mg) and high (400 mg) dose consumed in the morning (12 h prior to bedtime), afternoon (eight hours prior to bedtime), and evening (four hours prior to bedtime). We found no significant effect of the low or high theacrine dose on subsequent sleep although the high dose showed small non-significant effects on sleep efficiency and wake after sleep onset at each timepoint of consumption. However, consuming theacrine within eight hours of bedtime improved next-morning cognitive performance, with the 400 mg dose reducing the number of lapses on the Psychomotor Vigilance Task, although there were no significant effects on reaction time. Our findings provide initial scientific evidence suggesting that theacrine consumption may improve some aspects of next-morning cognitive performance but not others, with small non-significant effects on nighttime sleep.

Keywords Caffeine, Adenosine, Sleepiness, Sleep disruption, Vigilance, Alertness

Psychostimulants are often consumed to counteract decrements in cognitive function caused by insufficient sleep[1]. Of these, arguably the most commonly used psychostimulant is caffeine, being consumed by approximately 80% of the population[2,3]. Through its action as an adenosine antagonist, caffeine can alter homeostatic regulation of sleep and wake to increase arousal and reduce perceptions of fatigue[4]. Accordingly, caffeine is consumed through both natural (e.g., coffee) and synthetic sources (e.g., No-Doz) to improve cognitive performance[5], with a demonstrated ability to increase alertness, vigilance, and reaction time[6]. Despite its efficacy in improving cognitive performance, consumption of caffeine presents with several unwanted side effects including anxiousness, tachycardia, and sleep disturbance[5,7]. As such, there has been interest in identifying alternative supplements that enhance cognitive performance without compromising mood, heart health, and sleep quality. Theacrine has recently been proposed as one such alternative.

Theacrine is a purine alkaloid metabolised from caffeine in the leaves of various plants with highest abundance in *Camellia assamica var. kucha* and *Coffea liberica*[8]. With a similar molecular structure to caffeine, it is proposed that theacrine acts on adenosine receptors in the central nervous system in a manner similar to that of caffeine[9]. However, theacrine has a markedly different pharmacokinetic profile. While caffeine typically reaches its peak plasma concentration in one hour, theacrine reaches its peak two hours after consumption[10,11]. Additionally, theacrine has a substantially longer half-life of 16 to 26 h compared to the typical three to six hour half-life of caffeine[10,12]. Noting the different pharmacokinetic profile and duration of presence, as well as other potential

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physiological attributes, it is proposed that theacrine may improve cognitive performance in a similar manner to caffeine, but without the adverse side-effects[13].

Initial investigations on theacrine have primarily focused on its safety profile. Daily intake of up to 300 mg of theacrine across eight weeks had no negative effect on blood pressure, heart rate, electrocardiogram indicators of cardiac function, or blood safety markers in sixty healthy adults[14]. Similarly, the intake of 200 mg across seven days had no negative effect on heart rate, blood pressure, or gas exchange in fifteen healthy adults[15]. Despite the emerging interest in theacrine as an alternative to caffeine, there is limited evidence available regarding its cognitive and behavioural effects. The initial data support a positive effect on self-reported arousal[14–16], with objective improvements in cognitive performance observed only when theacrine is consumed in a supplement blend containing caffeine[17,18]. Furthermore, there is no evidence currently available regarding the effect of theacrine on subsequent sleep. This is an important consideration given sleep disruption in habitual caffeine consumers may perpetuate a cycle of insufficient sleep and subsequent caffeine reliance[19]. Therefore, this study aims to: 1) investigate the effect of theacrine dose and timing combinations on cognitive performance across the day of consumption and the following morning (next-day performance) and 2) investigate the effect of theacrine dose and timing combinations on the characteristics of subsequent night-time sleep.

Materials and methods

Participants

A sample of 21 participants was required. Using G*Power statistical software, the power calculation was based on a small effect size difference (Cohen's $f = 0.2$), an alpha level of 0.05, and 80% power. Given the lack of published research on the effect of theacrine on sleep, the effect size difference was used based on the effect of caffeine on total sleep time[7]. A correlation value of 0.60 was used to account for the repeated measures within individuals. This correlation value was based on the known effect of caffeine on the primary outcome variable of total sleep time, which was estimated using the raw data from Robillard et al.[20]. Participants were ineligible if in the three months prior to study admission they had used cigarettes, drugs, or medications known to affect sleep or had undertaken overnight shift work or transmeridian travel. While the screening process aimed to identify factors influencing sleep, participants were not explicitly asked about their use of over-the-counter sleep aids. General health was assessed using the 12-Item General Health Questionnaire[21] with $\geq 85\%$ of responses required to match the specified criteria to be eligible. Sleep health was assessed using the Pittsburgh Sleep Quality Index with a score of ≤ 5 required to be eligible. Each participant provided informed, written consent prior to participation and received financial compensation.

Experimental design and protocol

The study was approved by the Australian Catholic University (ACU) Human Research Ethics Committee (2023-3103HC) and registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12623000308695), with all research performed in accordance with the relevant guidelines and regulations. Using a placebo-controlled, double-blind, randomised crossover design, participants completed seven conditions including a placebo (glucose powder; Inova Pharmaceuticals, Australia) and 100 and 400 mg of theacrine (<99% purity synthetic powder; Health Sources, China) administered at 12, eight, and four hours prior to bedtime using identical appearing, weight-matched cellulose capsules. The doses chosen were based off a typical dose of caffeine (100 mg)[22] and the recommended upper daily limit of caffeine (400 mg)[5], with the timings designed to reflect morning, afternoon, and evening consumption. Each condition was separated by a six-day washout period to account for the half-life of theacrine[10]. Conditions were randomly assigned an alphabetical identifier from A to G and a condition sequence was constructed using a random number generator (i.e., 1 = A through to G = 7). The condition sequences were organised using a Latin square design. Each participant was randomly allocated to a condition sequence upon enrolment in the study. Generation and allocation of sequences was performed by an independent researcher using an online random number generator (RANDOM.ORG, Ireland). The protocol involved a seven-day baseline period followed by a 45-day intervention period completed in the participants' home environments. No changes were made to the experimental design or the outcomes of interest after trial commencement.

Baseline monitoring

During the seven-day baseline monitoring, participants wore an activity monitor (Spectrum Plus; Phillips Respironics, United States), completed a daily electronic sleep diary via the Research Electronic Data Capture (REDCap) tool hosted at ACU[23], and were required to maintain consistent bed and wake times (± 30 min). The baseline data were used to calculate each participant's average bed and wake times to inform an individualised intervention protocol, including a tailored sleep and wake schedule. Compliance with these scheduled bed and wake times is presented in Table 1. Across the baseline monitoring period, participants completed a validated caffeine intake questionnaire[23], previously employed with similar populations[24,25], to quantify habitual caffeine intake.

Intervention period

For the entirety of the intervention period, participants refrained from consuming Kucha tea or theacrine-containing supplements to prevent additional theacrine intake. On the day prior to and the day of each condition, protocols to control caffeine intake, alcohol intake, and napping were implemented. As caffeine abstinence may be a confounding factor with potential withdrawal effects[26,27], participants consumed a moderate dose ($3\ mg{\cdot}kg^{-1}$) of caffeine within 30 to 60 min of waking and refrained from consuming additional sources of caffeine. Additionally, participants refrained from consuming alcohol or napping on these days. Compliance was assessed using an electronic diet diary (Easy Diet Diary; Xyris Software, Australia).

The intervention period involved seven condition days, each preceded by a control day, and separated by a six-day washout period (Fig. 1). During this time, participants were requested to maintain a consistent bed and wake time (±30 min), wear an activity monitor, and complete a daily sleep diary to monitor protocol compliance. On each condition day, participants consumed a capsule at 12, eight, and four hours prior to their bedtime, individualised from the baseline monitoring. For the theacrine conditions, one capsule contained the theacrine dose (either 100 or 400 mg) and the remaining two capsules contained the placebo, while all capsules contained the placebo for the control condition. Each participant was provided a capsule case clearly identifying the order, day, and time of each capsule's consumption. Participants received a text-message reminder to their phone to consume the specified capsule and compliance was confirmed with a return message.

Measures

Objective sleep

A validated partial polysomnography device (Somfit; Compumedics, Australia) measured sleep[28]. Participants attached the device to their forehead using an electrode patch and controlled the recording via the smartphone application. Data were scored by the proprietary algorithm to provide total sleep time, sleep efficiency (total sleep time/time in bed × 100), sleep onset latency (time from lights out to the first sleep epoch), latency to persistent sleep (time from lights out to the first 10 min of consecutive sleep epochs), rapid eye movement (REM) sleep onset latency (time from lights out to the first REM sleep epoch), wake after sleep onset (total amount of time spent awake following sleep onset), number of awakenings (transition from a sleep epoch to a wake epoch) per hour, and sleep architecture (non-rapid eye movement (NREM) stage one (N1) and stage two (N2) (light sleep), NREM stage three (N3), and REM sleep).

Subjective sleep

Participants completed an electronic sleep diary within 30 min of waking to provide subjective total sleep time (hours), sleep onset latency (minutes), wake after sleep onset (minutes), sleep quality (1–5 Likert scale anchored by "1" as equivalent to "very poor" and "5" as equivalent to "very good"), and pre-sleep alertness (1–5 Likert scale anchored by "1" as equivalent to "none" and "5" as equivalent to "very high"). Immediately prior to bedtime participants indicated their acute level of sleepiness using the Karolinska Sleepiness Scale, a nine-point Likert scale anchored by "1" as equivalent to "extremely alert" and "9" as equivalent to "very sleepy".

Perceived level of arousal

Participants completed five electronic visual analogue scales 15 min post waking, 10, six, and two hours prior to bedtime, and 15 min post waking the following morning to rate level of fatigue, anxiousness, alertness, overall positivity of mood, and readiness to perform on upcoming cognitive tasks. Each visual analogue scale was anchored by "none at all" (0) and "maximal" (100).

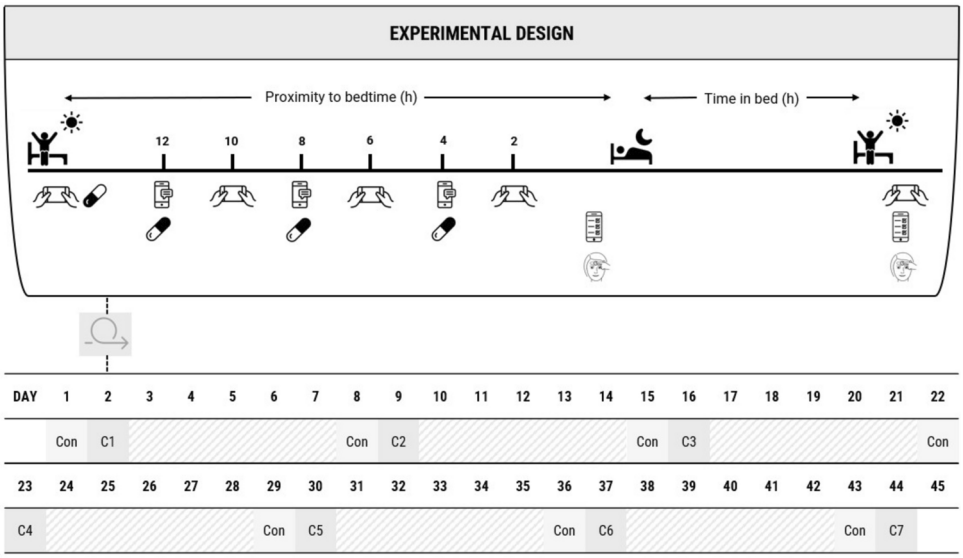

Fig. 1. Experimental phase of the protocol. Participants completed seven conditions (C1-C7) in a randomised manner with a six-day washout period between each. On each control day (con) and condition day, participants consumed a standardised dose of caffeine (3 mg·kg^{-1}) within 30 to 60 min of waking in capsule form. Three capsules (placebo or theacrine) were administered at 12, eight, and four hours prior to bedtime in line with the assigned condition. Participants completed a set of five visual analogue scales followed by the Psychomotor Vigilance Task and Simon task 15 min post waking, 10, six, and two hours prior to bedtime, and 15 min post waking the following day. Immediately prior to bedtime, participants applied the Somfit device and completed the Karolinska Sleepiness Scale. Upon waking, participants removed the Somfit device, recorded their perception of the condition, and completed the sleep diary.

Cognitive testing
A three-minute PVT and two-minute Simon task were completed immediately following the visual analogue scales. Participants completed each task on their smartphone (Inquist6; Millisecond, United States).

Psychomotor Vigilance Task
The PVT assessed the cognitive constructs of sustained attention and vigilance[29]. Participants reacted to visual stimuli that randomly appeared on the device screen with an interstimulus interval between two and 10 s. Outcomes included mean reaction time (average duration of responses with a latency ≤ 500 ms), number of lapses (transformed number of responses with a latency > 500 ms), and range reaction time (average duration of responses between the 10^{th} and 90^{th} percentile of responses with a latency ≤ 500 ms).

Simon Task
The Simon task assessed the cognitive constructs of response selection and response inhibition[30]. Participants reacted to a visual stimulus that appeared on either the left or right side of the device screen. For congruent trials, the spatial location of the stimulus matched the response location and for incongruent trials, the spatial position conflicted with the correct response. Outcomes included the proportion (%) of correct responses for all trials, congruent trials, and incongruent trials, the response time (ms) of correct responses for all trials, congruent trials, and incongruent trials, and the Simon effect (mean difference between the average response time of congruent and incongruent trials).

Perception of condition
Participants completed an electronic questionnaire within 30 min of waking the morning following a condition to indicate whether they believed they had consumed theacrine, and if so, at what dose they believed this occurred.

Statistical analysis
Statistical analyses were performed in R (V4.3.1, R Foundation for Statistical Computing, Austria). Linear mixed models were built using the *lmerTest::lmer* function to examine the effects of theacrine dose and timing on cognitive performance and sleep. Models included theacrine condition as a fixed effect and a random intercept of participant ID to adjust for non-independence. If the condition term in the omnibus ANOVA was significant, then planned estimated marginal means post-hoc analyses were used to examine significant changes in cognitive performance and sleep as a function of condition using the *emmeans::emmeans* and *emmeans::contrasts* functions. Five sets of contrasts (15 contrasts per analysis) compared each theacrine dose (and placebo) within each timepoint (three sets of three contrasts) and each timepoint within each theacrine dose (three sets of two contrasts). We adjusted for multiple comparisons using a Bonferroni familywise adjustment within each set of contrasts. To account for the repeated measures among individuals and estimate appropriately weighted effect sizes (Cohen's *d*) and 95% confidence intervals were estimated from the t statistic in the mixed effects models using the effectsize::t_to_d function. Effect size differences were interpreted as trivial; < 0.20, small;0.20–0.49, medium;0.50–0.79, and large; ≥ 0.8. Data are reported as means ± standard deviation (SD) with statistical significance set to $p < 0.050$. Descriptive statistics are presented in Supplementary Table S1 to S4 and comparisons in Supplementary Table S5 to S9.

Results
Participants
Twenty-four participants were enrolled in the study between July 2023 and November 2023. Prior to the intervention, two participants withdrew from the study due to the required time commitment. Therefore, 22 healthy males (age: 25.0 ± 4.6 years; height: 182.4 ± 8.0 cm; and mass: 88.0 ± 15.2 kg) with a moderate habitual caffeine intake (136.7 ± 82.5 mg·day^{-1}) completed the study. Data collection concluded in February 2024 upon successful completion of the required sample size. No adverse events were reported during the data collection.

Objective sleep
There were no significant differences in bedtime, waketime, and time in bed across conditions ($p > 0.050$). There was a significant main effect on wake after sleep onset ($p = 014$), sleep efficiency ($p = 0.012$), and awakenings per hour ($p = 0.042$).

Placebo contrasts
Post-hoc comparisons showed no significant effects of either the 100 or 400 mg dose of theacrine on objective sleep when compared to the placebo at each timepoint (Fig. 2). The 400 mg dose of theacrine had a small, non-significant effect on sleep efficiency when consumed four (-5.4%;$p = 0.087$; $d = -0.22$ [-0.46;0.02]), eight (-5.5%; $p = 0.091$; $d = -0.22$ [-0.46;0.02]), and 12 (-4.7%; $p = 0.154$; $d = -0.20$ [-0.44;0.05]) hours prior to bedtime, on wake after sleep onset when consumed four (+21.3 min; $p = 0.142$; $d = 0.20$ [-0.04;0.44]), eight (+23.5 min; $p = 0.094$; $d = 0.22$ [-0.02;0.45]), and 12 (+24.1 min; $p = 0.062$; $d = 0.23$ [-0.01;0.48]) hours prior to bedtime, and on awakenings per hour when consumed four (+1.2 count; $p = 0.090$; $d = 0.22$ [-0.02;0.46]) and eight hours prior to bedtime (+1.3 count; $p = 0.077$; $d = 0.23$ [-0.02;0.46]).

Dose and timing contrasts
The 400 mg dose of theacrine had a significant effect on certain sleep characteristics compared to the 100 mg dose of theacrine. Consumption eight hours prior to bedtime reduced sleep efficiency (-7.5%; $p = 0.015$; $d = -0.29$ [-0.53;-0.04]) and increased wake after sleep onset (+29.8 min; $p = 0.027$; $d = 0.26$ [0.02;0.51]) and the number of awakenings per hour (+1.5; $p = 0.041$; $d = 0.25$ [0.01;0.49]). In addition, compared to the 100 mg dose, the

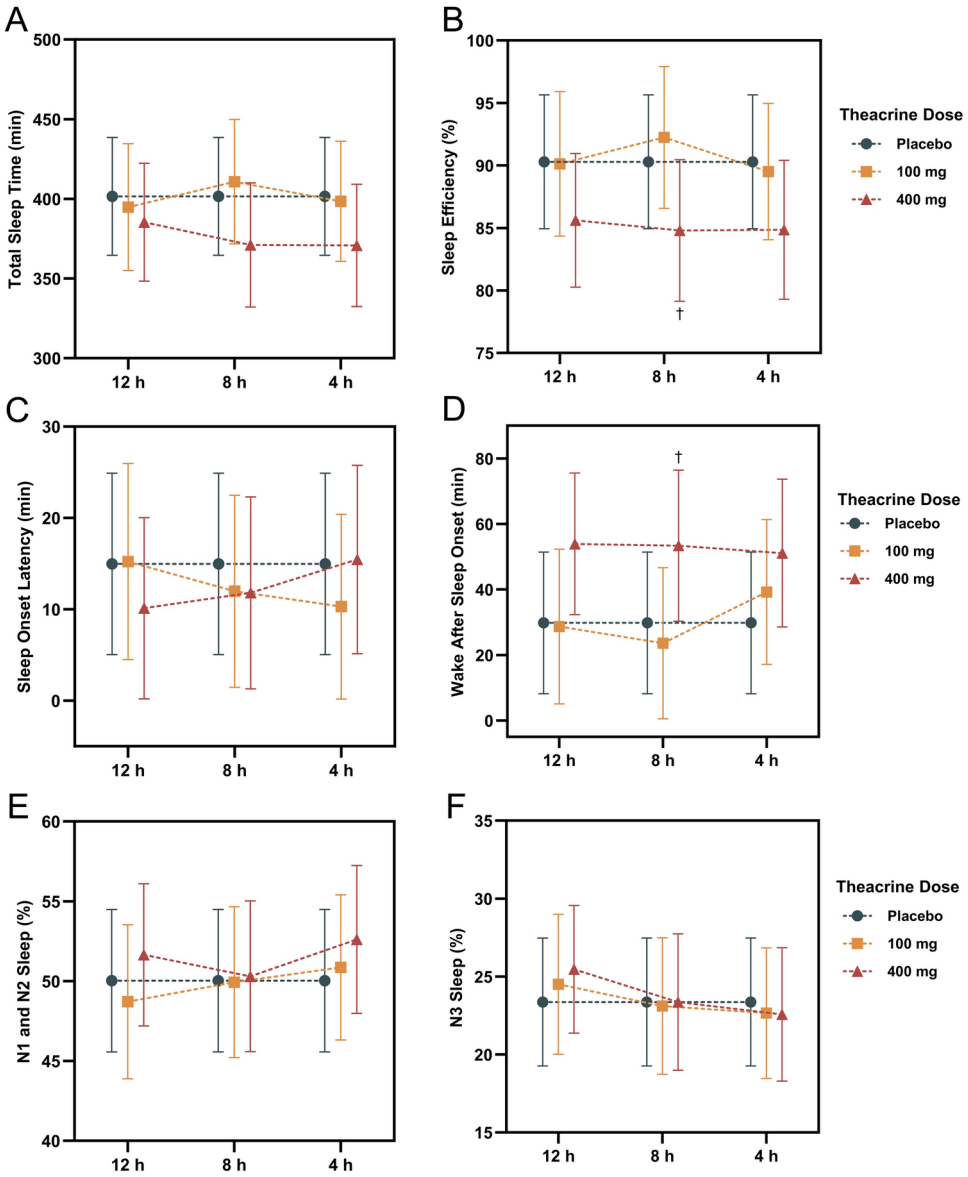

Fig. 2. Change in objective sleep measures: (**A**) total sleep time (minutes); (**B**) sleep efficiency (%); (**C**) sleep onset latency (minutes); (**D**) wake after sleep onset (minutes); (**E**) proportion of N1 & N2 sleep (%); and (**F**) proportion of N3 sleep (%) for placebo, 100 mg of theacrine, and 400 mg of theacrine consumed 12, eight, and four hours prior to bedtime. †indicates a significant difference compared to the 100 mg dose of theacrine within the same timepoint. Error bars represent adjusted 95% confidence intervals.

400 mg dose of theacrine had a small, non-significant effect on wake after sleep onset (+25.3 min; $p = 0.070$; $d = -0.23$ [-0.01;0.47]) when consumed 12 h prior to bedtime. No significant timing effects were observed on objective sleep for the 100 or 400 mg dose of theacrine ($p > 0.050$).

Subjective sleep

There was a significant main effect on perceived sleep onset latency ($p = 0.036$). The main effects for the remaining subjective sleep outcomes were not significant ($p > 0.050$).

Placebo contrasts

The 400 mg dose of theacrine consumed 12 h prior to bedtime significantly increased perceived sleep onset latency (+18.5 min; $p = 0.029$; $d = 0.27$ [0.02;0.52]) compared to the placebo (Fig. 3). No significant effects were observed on subjective sleep with the 100 mg dose of theacrine compared to the placebo ($p > 0.050$).

Dose and timing contrasts

There was no significant effect on subjective sleep when comparing the 100 and 400 mg dose of theacrine at each timepoint ($p > 0.050$). For the 400 mg dose, consumption eight hours prior to bedtime significantly reduced

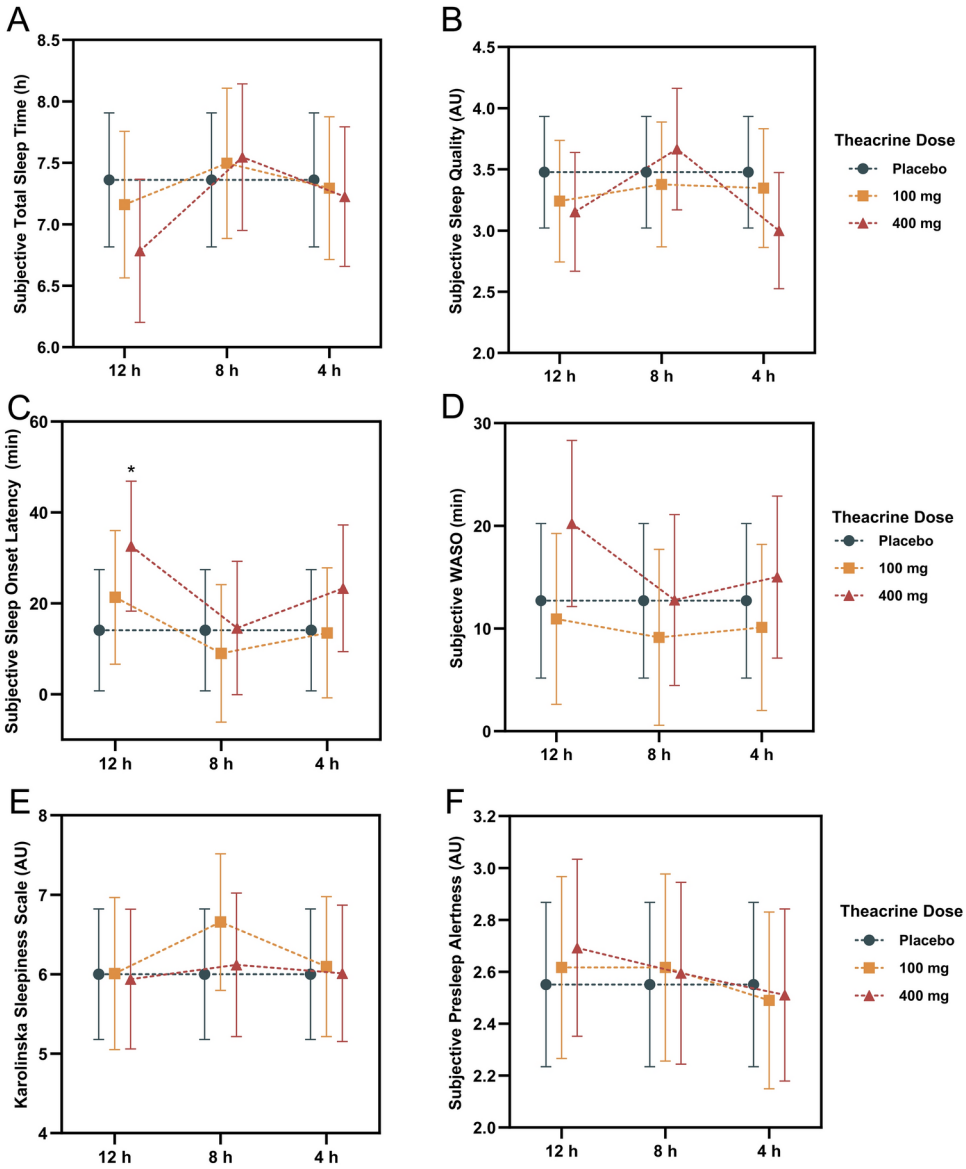

Fig. 3. Change in subjective sleep measures: (**A**) total sleep time (minutes); (**B**) sleep quality (units); (**C**) sleep onset latency (minutes); (**D**) wake after sleep onset (WASO) (minutes); (**E**) Karolinska Sleepiness Scale (units); and (**F**) pre-sleep alertness (units) for placebo, 100 mg of theacrine, and 400 mg of theacrine consumed 12, eight, and four hours prior to bedtime. *indicates a significant difference to the placebo within the same timepoint. Error bars represent adjusted 95% confidence intervals.

perceived sleep onset latency (-18.0 min; $p = 0.049$; $d = -0.25$ [-0.51;0.00]) compared to consumption at 12 h. No timing effect was observed for the 100 mg dose ($p > 0.050$).

Perceived level of arousal

No significant main effects were observed on perceived level of arousal ($p > 0.050$). Therefore, post-hoc comparisons were not reported.

Psychomotor Vigilance Task

There was a significant main effect on the number of transformed lapses when assessed 15 min post wake the next morning ($p = 0.025$), while the main effects for remaining PVT outcomes were not significant ($p > 0.050$).

Placebo contrasts

For testing 15 min post wake the next morning, the 400 mg dose of theacrine significantly reduced the number of lapses when consumed four (-1.2; $p = 0.009$; $d = -0.31$ [-0.55;-0.06]) and eight (-1.1; $p = 0.027$; $d = -0.27$ [-0.51;-0.02]) hours prior to bedtime compared to the placebo (Fig. 4). No significant effects were observed with the 100 mg dose of theacrine compared to the placebo ($p > 0.050$).

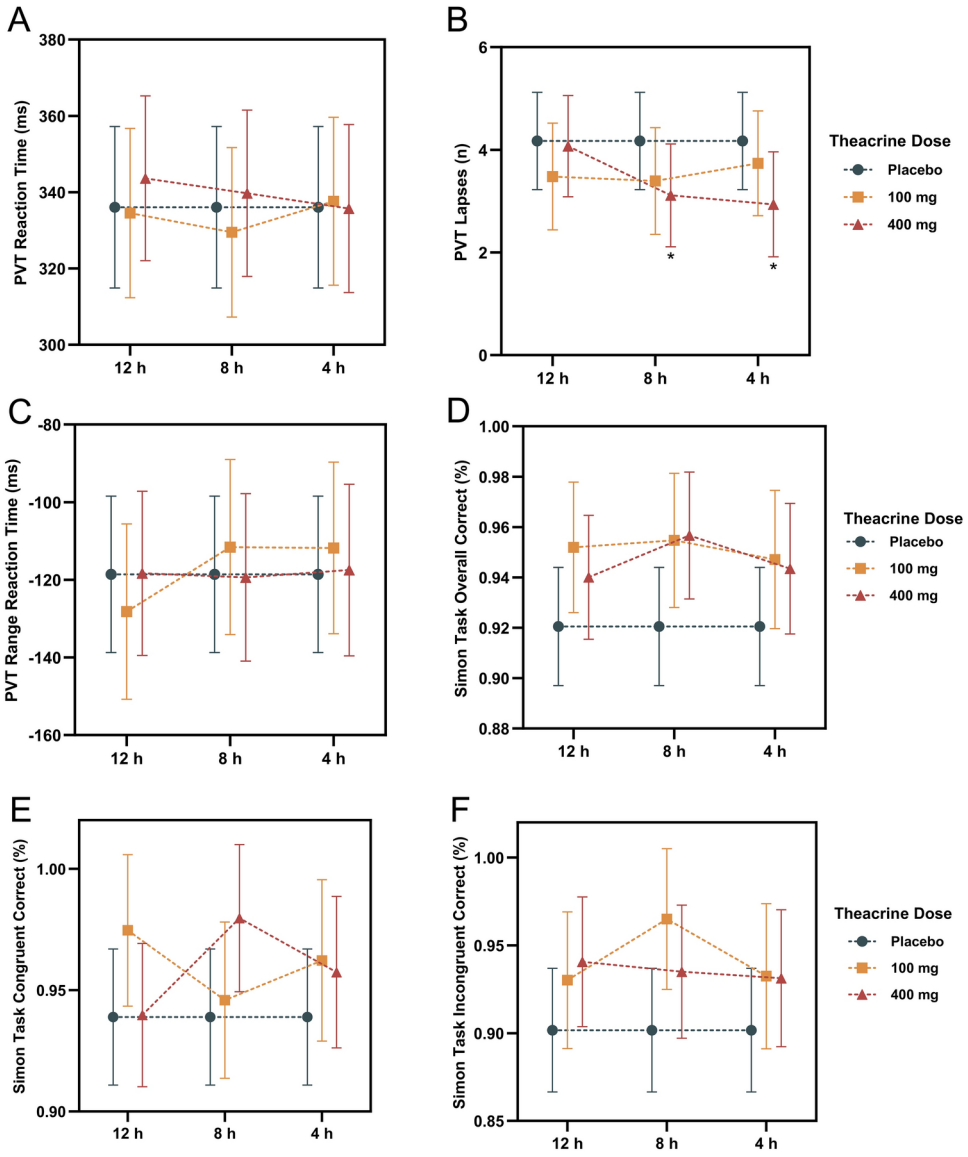

Fig. 4. Change in cognitive performance outcomes assessed 15-min upon waking the following morning: (**A**) Psychomotor Vigilance Task (PVT) mean reaction time (ms); (**B**) PVT number of lapses (count); (**C**) PVT range mean reaction time (ms); (**D**) Simon task overall proportion of correct responses (%); (**E**) Simon task congruent proportion of correct responses (%); and (**F**) Simon task incongruent proportion of correct responses (%) for placebo, 100 mg of theacrine, and 400 mg of theacrine consumed 12, eight, and four hours prior to bedtime. *indicates a significant difference to the placebo within the same timepoint. Error bars represent adjusted 95% confidence intervals.

Dose and timing contrasts

No significant effect was observed on PVT outcomes between the 100 and 400 mg dose of theacrine at each timepoint ($p > 0.050$). The 400 mg dose of theacrine consumed four hours prior to bedtime significantly reduced next-morning number of lapses when compared to consumption at 12 h (-1.1; $p = 0.026$; $d = -0.27$ [-0.51;-0.02]), with a small non-significant reduction in next-morning number of lapses when consumed at eight hours compared to 12 h (-1.0; $p = 0.071$; $d = -0.23$ [-0.48;-0.01]). No timing effect was observed for the 100 mg dose ($p > 0.050$).

Simon task

There were no significant main effects observed for Simon task outcomes ($p > 0.050$). Therefore, post-hoc comparisons were not reported.

Perception of condition

When asked to state which dose and the timing of that dose they received, 36% of participants identified the correct dose, 25% identified the correct timing of consumption, and 13% identified the correct dose and timing across conditions.

Discussion

This study investigated the effect of the dose and timing of theacrine intake on cognitive performance and subsequent sleep. Conditions were designed to replicate a low and high dose of theacrine in the morning, afternoon, and evening, while controlling for a moderate intake of caffeine. The key findings are: 1) Theacrine consumption had no significant effect on objective sleep compared to the placebo, regardless of the dose or timing. However, the 400 mg dose of theacrine negatively affected certain sleep characteristics compared to the 100 mg dose; 2) Subjective sleep and perceived arousal were largely unaffected by theacrine consumption; and 3) Aspects of next-morning cognitive performance were enhanced by consuming 400 mg of theacrine. Overall, the findings suggest that while theacrine may not acutely improve cognitive performance, it may improve next-morning cognitive performance without significantly impacting night-time sleep (summary of effects displayed in Fig. 5).

The consumption of theacrine had no significant impact on objective sleep compared to placebo. There was a small, non-significant increase in wake after sleep onset of ~ 20 min and reduction in sleep efficiency of ~ 5% at each timepoint of consumption for the 400 mg dose of theacrine. With a change of 20 min in wake after sleep onset and 5% in sleep efficiency indicating clinically meaningful change[31], further investigation is required to assess whether a high dose of theacrine increases subsequent sleep fragmentation. In contrast, sleep disruption

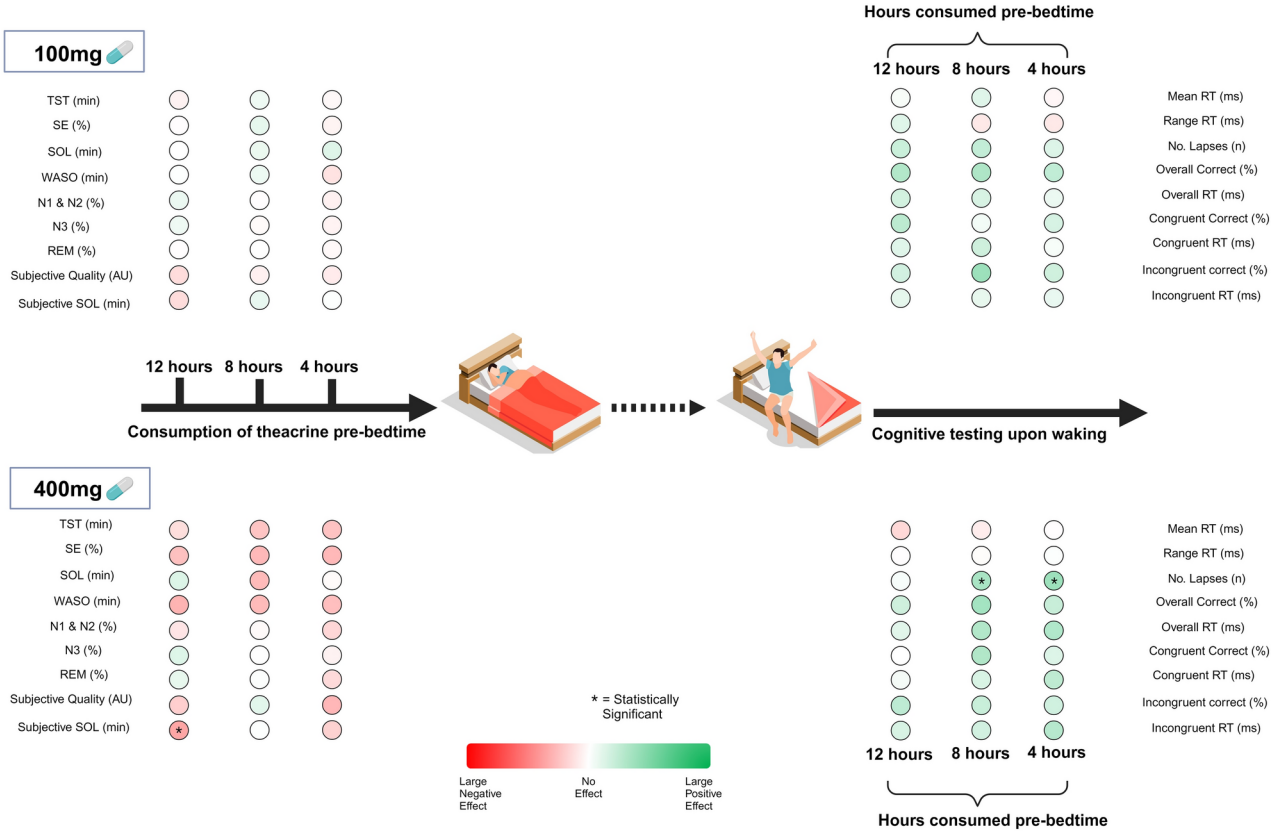

Fig. 5. A summary of the effects of 100 mg of theacrine and 400 mg of theacrine consumed 12, eight, and four hours prior to bedtime on subsequent sleep outcomes (left) of total sleep time (TST), sleep efficiency (SE), sleep onset latency (SOL), wake after sleep onset (WASO), non-rapid eye movement (NREM) stage one (N1) and two (N2) sleep, NREM stage three (N3) sleep, rapid eye movement (REM) sleep, subjective sleep quality, and subjective SOL and on cognitive perfomance assessed 15 min upon waking the following morning for outcomes (right) of Psychomotor Vigilance Task (PVT) mean reaction time (mean RT), PVT range mean reaction time (range RT); PVT number of lapses (no. lapses), Simon task overall proportion of correct responses and mean reaction time of responses (overall correct and overall RT, respectively), Simon task congruent proportion of correct responses and mean reaction time of responses (congruent correct and congruent RT, respectively), and Simon task incongruent proportion of correct responses and mean reaction time of responses (incongruent correct and incongruent RT, respectively. The effect size scale ranges from -0.8 to 0.8 (large effect), with a red effect indicating a negative influence on the sleep or cognitive performance outcome and a green effect indicating a positive influence on the sleep or cognitive outcome.

is a well characterised side effect of caffeine when consumed in doses up to 400 mg, with significantly poorer total sleep time, sleep efficiency, wake after sleep onset, and sleep onset latency compared to the placebo[7]. The negative effect of caffeine on sleep is typically greater with a closer consumption to bedtime[7]. The findings of the present study suggest that theacrine, unlike caffeine, does not significantly impact subsequent sleep, regardless of the dose or timing of consumption.

Sleep architecture was not significantly altered with the consumption of theacrine. This is an interesting finding given caffeine consumption typically increases the occurrence of N1 & N2 sleep (i.e., light sleep) at the expense of N3 sleep (i.e., deep sleep)[7]. The expression of N3 sleep is suggested to be under homeostatic control, with these changes in sleep architecture linked to a reduction in homeostatic sleep pressure[32]. As the accumulation of extracellular adenosine increases homeostatic sleep pressure, the action of caffeine in blocking adenosine binding promotes wakefulness[33]. Despite the similarity in molecular structure between theacrine and caffeine, it remains unclear whether theacrine acts on the central nervous system as an adenosine antagonist or an allosteric modulator to dampen adenosine signalling[9,18]. The divergence in the effects on sleep architecture suggests that theacrine may influence the central nervous system through a different mechanism to caffeine. However, further research is needed to investigate the mechanistic action of theacrine. Overall, the findings of the present study suggest that theacrine does not disrupt subsequent sleep architecture in the same manner as caffeine.

Although there were no significant effects on objective sleep when theacrine was compared to the placebo, the 400 mg dose of theacrine had a negative impact when compared to the 100 mg dose. This finding might be explained by the separate hypothesis that theacrine has opposite effects on sleep depending on the dose, with evidence from rodent studies suggesting theacrine acts as a hypnotic when consumed at a low dose[34,35]. For example, when rodents were administered a low dose of theacrine, there was a significant increase in total sleep time compared to the control, while those treated with the same dose of caffeine had significantly reduced total sleep time[35]. Additionally, in a separate rodent study, administration of a low dose of theacrine significantly reduced sleep onset latency and increased total sleep time compared to the control, leading the authors to propose theacrine as a promising treatment for insomnia[34]. However, in the present study, there was no evidence of improvements in objective sleep when comparing the 100 mg dose of theacrine to the placebo. Although the present findings show an intriguing dose-dependent effect of theacrine on objective sleep, this is the first study to evaluate this effect in humans and further scientific research is required to build on these findings.

Perceptions of sleep and arousal were largely unaffected by the consumption of theacrine. Participants perceived a significantly longer sleep onset latency when consuming the 400 mg dose of theacrine 12 h prior to bedtime compared to the placebo, despite no significant effect when assessed objectively. There is evidence to suggest that the time of day of at which theacrine is consumed may alter the perceived effect of the substance[36–38]. Previous studies investigating theacrine have shown mixed effects on self-reported level of arousal. For example, an acute dose of 200 mg significantly increased energy and reduced fatigue when assessed using visual analogue scales[15], while the acute intake of 300 mg had no significant effect on arousal when assessed using visual analogue scales[39] or an adapted Profile of Moods States questionnaire[14]. Comparatively, the intake of 100 to 400 mg of caffeine typically results in positive impacts on markers of perceived arousal[40], with subsequent reports of poor subjective sleep outcomes[7]. The findings of the present study suggest that perceptions of sleep and arousal are largely unaffected by the consumption of 100 or 400 mg of theacrine.

Objective indicators of improved cognitive performance were observed the morning following theacrine consumption. Specifically, 400 mg of theacrine significantly reduced the number of lapses on the PVT when consumed four and eight hours prior to bedtime, indicating a positive effect on sustained attention[29]. Similar positive effects on PVT performance have been observed with caffeine doses ranging from 80 to 400 mg in the hours immediately following consumption[6,41]. However, the positive impact of theacrine on cognitive performance was observed approximately 12 to 16 h post consumption. The difference in the time-course of the effect may be attributed to the difference in half-life, with that of theacrine (16 to 26 h)[10] approximately five times longer than caffeine (three to six hours)[10,12]. Overall, the findings suggest that theacrine consumption may positively impact aspects of cognitive performance, although consumption may need to occur substantially earlier relative to the performance task compared to caffeine.

Limitations

Although this study provides novel insights into the utility of theacrine, there are several limitations that should be considered when interpreting the findings. First, plasma concentrations of theacrine were not measured, limiting the ability to evaluate the relationship between the time-course of theacrine plasma concentration, sleep, and cognitive performance. Additionally, as caffeine intake was standardised on condition days, our findings present the effect of theacrine in the presence of morning caffeine intake, which may increase the bioavailability of theacrine[10]. Future investigations should consider alternative approaches to address habitual caffeine intake and potential withdrawal that allows consumption of theacrine independent of caffeine. It is well supported that caffeine half-life and effect can be influenced by genetic predispositions[42,43]. With suggestions that theacrine is metabolised through the same pathway as caffeine and may act similarly on adenosine receptors in the brain, it is reasonable that future work should investigate the moderating influence of single nucleotide polymorphisms in key genes including *CYP1A2* variant rs762551 and *ADORA2A* variant rs5751876. Second, our present study was conducted in a naturalistic setting, and although this increases the ecological validity of the findings, it limits the ability to control external influences that may confound the results. Despite the use of a randomised, cross-over design, common factors including dietary intake (e.g., macronutrient composition and timing of intake), environmental factors (e.g., noise and temperature), and standardisation of performance testing (e.g., location and body position) may have had a confounding influence and would typically be controlled in a laboratory setting[44]. Third, the study employed healthy, young, male adults and the findings may not be generalisable to

other populations. Lastly, although the Somfit has been validated[28], it is recommended to be used to assess meaningful changes in sleep architecture over time, and caution may need to be applied with one measurement time-point for each condition. In addition, detection of sleep onset latency can be challenging outside of a laboratory based polysomnography assessment. Although it is common for discrepancies in subjective and objective assessments of sleep[45], the discrepancy between sleep onset latency outcomes reported in this study may be a result of the method used to detect sleep onset latency.

Future recommendations

The present study provides evidence to suggest theacrine consumption does not disrupt subsequent sleep in the same manner as caffeine. It is important to note that small, non-significant effects on sleep were observed with the 400 mg dose and further scientific research is required to draw firm conclusions on the impact of theacrine on subsequent sleep. With this, an effort to investigate the mechanism of action of theacrine is necessary, with consideration of how theacrine may impact sleep–wake regulation. Additionally, there was a positive effect on objective indicators of cognitive performance, although the time-course for this effect is substantially longer than that of caffeine. Therefore, further scientific investigations regarding the optimal dose and timing of theacrine intake relative to the cognitive performance task is recommended. Given the extended time-course of the effect of theacrine, it would be worthwhile investigating the effect on cognitive performance and sleep across multiple days, with protocols that involve both acute and chronic administration. Finally, when considering the efficacy of theacrine as an alternative to caffeine, it would be useful to include caffeine as a condition in the experimental design to allow comparison. Such research should consider the potential for a theacrine and caffeine combination, with the synergistic effect having potential to offer improvements to cognitive performance while minimising subsequent sleep disruption.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Received: 21 June 2024; Accepted: 6 November 2024
Published online: 19 November 2024

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Author contributions

CG, SH, JW, LB, LGK, and SR conceptualised the study. CG, SH, JW, and SR contributed to data acquisition and analysis. JL and RJ conducted statistical analysis. CG, SH, and JW drafted the manuscript, which was revised by LB, LGK, SR, JL, RJ, and AT. All authors approved the final version of the manuscript.

Declarations

Competing interests

Dr. Karagounis has received compensation as a consultant for RNWY. The remaining authors declare no competing interests and the study received no external funding.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-79046-2.

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