KU LEUVEN

FACULTY OF PSYCHOLOGY AND
EDUCATIONAL SCIENCES

The Effects of Caffeine on Procedural Memory

A Psychopharmacological Study with Human Subjects

Master's thesis submitted for the degree of
Master of Science in Master of
Psychology: Theory and Research by
Alexandre Spacassassi Silva

Supervisor: Prof. Dr. Rudi D'Hooge

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Summary

While caffeine is known to enhance basic cognitive functions such as attention, vigilance, and reaction time, its influence on long-term memory is still a subject of debate. It is particularly unclear whether caffeine affects procedural memory, a type of long-term memory that stores information related to motor skills, habits, and actions, such as riding a bicycle or tying shoes. Elucidating this relation could be vital due to the crucial role of procedural memory in the acquisition of new skills and caffeine's widespread consumption. For instance, if caffeine is detrimental to the acquisition of procedural information, then its use should be limited in those with procedural memory deficits, such as individuals with Parkinson's disease. On the other hand, caffeine might be beneficial for procedural learning, raising the possibility of utilizing caffeine strategically to enhance learning.

This study examined whether post-learning caffeine ingestion enhances procedural memory consolidation using a double-blind, placebo-controlled, mixed design. Procedural memory consolidation was assessed via performance on a serial reaction time task (SRTT). Thirty healthy adults were randomly assigned to receive either a placebo, 80mg, or 240mg of caffeine following initial SRTT completion. Performance was reassessed 24 hours later, and the difference in performance between days provided a measure of consolidation. Trial-level reaction time (RT) and accuracy were analysed using generalized linear mixed-effects models and aligned rank transform ANOVA. The analyses included covariates such as baseline affect, physical activity, and habitual caffeine use, while post-test affect and sleep duration were examined as potential mediators.

The results indicated that post-learning caffeine intake can improve procedural memory consolidation. Participants who received 80mg of caffeine showed greater RT improvement between sessions compared to placebo. In contrast, no such benefit was found in the 240 mg group, hinting at a diminished efficacy at higher doses. Higher habitual caffeine use predicted slower RT on day two, implying possible tolerance effects. Baseline positive affect was a significant predictor of faster RT, but post-test affect was not, suggesting caffeine's effects were not mediated by affect. The 80mg group exhibited the smallest changes in accuracy between sessions, indicating more stable performance across days.

In sum, moderate post-learning caffeine ingestion benefits procedural memory consolidation, reflected in both speed and accuracy improvements. However, the observed effects were modest, dose-dependent, and potentially diminished by habitual use. Therefore, caffeine's utility as a memory enhancer should be weighed carefully, particularly given its disrupting impact on sleep, a crucial factor in memory consolidation. Further research should clarify the conditions under which caffeine improves consolidation, including task complexity, individual differences, and timing of ingestion.

Acknowledgements

Over the course of two years, a figment of imagination has crossed the boundary of the mind into reality. However, the metamorphosis of this academic caterpillar, from conceptual cocoon to noisy actuality, could not have been achieved without the support of many others.

First, a word of gratitude towards my family, close and far, for their unwavering support throughout my entire journey as a student. Thank you for the home-cooked meals, the countless rides, the patience, and for reminding me that there is more to life than schoolwork. In particular, thank you to my grandparents for introducing me to coffee.

Thank you to my supervisor, Prof. Dr. D'Hooge, for believing in me, for giving me the opportunity to work on a self-proposed topic, for the shared enthusiasm in the project, and for the invaluable advice and feedback.

Thank you to my friends for helping me relax during stressful times, for understanding that I could not always be there, and for participating in my experiment. A special thanks to Sasha who ensured a controlled setting between sessions.

Finally, thank you to all the participants for enduring a boring experimental task not once, but twice. This work would not have been possible without them and their patience.

Clarification of Approach and Contribution

The topic of this thesis was self-proposed following an initial literature review that identified a gap regarding caffeine's effects on procedural memory. After approval, I defined the research methodology, sourced materials, and prepared the ethics application for Social and Societal Ethics Committee independently, with review and guidance provided by my supervisor, Prof. Dr. D'Hooge.

I conducted a focused second literature review to determine caffeine-related variables relevant to the research design. Procurement of both caffeine and placebo capsules, as well as sourcing and adapting the Serial Reaction Time Task code, was also completed independently, in consultation with my supervisor.

Participant recruitment, data collection, analysis, and writing were all carried out independently. The supervisor offered feedback primarily during the writing phase and advised on key decisions such as the need for a precise mode of caffeine administration, ethics approval, and the inclusion of affect as a covariate.

The decision to include physical activity as a covariate, as well as the selection of the International Physical Activity Questionnaire and the Positive and Negative Affect Schedule, was based on input from Dr. Anna M. Zamorano (Aarhus University).

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Introduction

Caffeine is the most widely consumed psychoactive substance in the world (James, 1997; Quadra et al., 2020). Its effects on physical and cognitive performance have been widely studied, with consistent evidence supporting its benefits for alertness, attention, and reaction time. However, caffeine's influence on long-term memory remains less clear as previous studies yielded mixed or inconclusive findings (McLellan et al., 2016; Nehlig, 2010). In particular, its effects on procedural memory, a form of long-term memory that supports the acquisition of motor skills, habits, and actions (Knowlton & Schorn, 2024), remains virtually unexplored. Nevertheless, understanding the relationship between caffeine and procedural memory is crucial for several reasons. First, procedural memory underlies a wide range of behaviours that rely on motor skill development (Cohen & Bacdayan, 1994; Doyon et al., 2018), including language learning (Stefaniak et al., 2021; Ullman, 2013). As such, the potential for caffeine to influence this type of memory has public health relevance, particularly given caffeine's widespread consumption. Clarifying this relationship could, for instance, inform evidence-based guidelines around caffeine use, balancing its possible cognitive benefits against risks, especially for populations with procedural memory impairments such as individuals with Parkinson's disease (Allain et al., 1995; Pauly et al., 2022) or Huntington's disease (Bylsma et al., 1990; Cayzac et al., 2011; Knopman & Nissen, 1991). Further, if caffeine enhances procedural memory, then it could be strategically leveraged in domains that rely on automatized skill performance, including sports (Iglesias et al., 2005), music (Baird & Samson, 2009; Cavaco et al., 2012), and high stress environments (Blacker et al., 2018). Finally, such findings could benefit physical rehabilitation programs since motor skill acquisition and consolidation are critical to functional recovery (Muratori et al., 2013; Siengsukon & Boyd, 2009; Stevans & Hall, 1998). To explore these possibilities, the present study investigates the effects of caffeine on procedural memory. The following sections outline background characteristics of caffeine relevant for interpreting its effects, followed by the study's methodology, findings, and an interpretation of results in light of prior literature.

Caffeine Consumption

Approximately 80% of the global population consumes caffeine on a daily basis, surpassing the consumption rates of other widely used psychoactive substances such as alcohol and nicotine (James, 1997). More recent estimates suggest that global caffeine consumption

continues to rise (Quadra et al., 2020), driven in part by the increased consumption of caffeinated soft drinks and energy drinks (Heckman et al., 2010). Notably, average caffeine consumption varies considerably between countries (see Table 1). For instance, the average per capita caffeine intake in France is estimated at 239mg per day, while in the Netherlands, it reaches 414mg (Fredholm et al., 1999). In comparison, the average intake in Belgium is considerably lower, ranging between 150 to 160mg (Superior Health Council, 2012).

These increases in caffeine consumption over time, combined with substantial cross-national variability in daily intake, pose a considerable challenge in defining a universal benchmark for what constitutes moderate caffeine consumption. This suggests that experimentally administered dosages should be tailored to specific populations and informed by the most recent data. For instance, national estimates, such as the 150 to 160mg per day reported for Belgium, are likely accurate within their local and temporal context, but may nonetheless underestimate current intake levels given the global upward trend in caffeine consumption.

Table 1

Caffeine Consumption in mg per Person per Day Across Countries

Country	Caffeine consumed	Primary source of caffeine
France	239	Coffee
Netherlands	414	Coffee
Paraguay	156	Yerba mate
Egypt	58	Tea
United States	168	Coffee
China	16	Tea

Note. Adapted with permission from "Actions of Caffeine in the Brain with Special Reference to Factors that Contribute to its Widespread use," by B. B. Fredholm, K. Bättig, J. Holmén, A. Nehlig & E. E. Zvartau, 1999, *Pharmacological Reviews, 51*(1), p. 85 (https://pubmed.ncbi.nlm.nih.gov/10049999). Copyright 1999 by Elsevier.

Caffeine's popularity is often attributed to its ubiquity, as the compound is naturally present in the beans, leaves, and fruits of more than 60 plant species. However, despite its widespread natural occurrence, caffeine is primarily sourced from just a few plants, most notably coffee and cacao beans, tea leaves, kola nuts, yerba mate, and guarana berries (Barone & Roberts,

1984; Barone & Roberts, 1996). In addition to its biological abundance, caffeine's widespread use is also deeply rooted in its long-standing relationship with human culture. Tea, for instance, has been consumed for thousands of years (Arab & Blumberg, 2008), and coffee has been a staple in various cultures for centuries (Kerr, 2021). Despite their distinct origins, both beverages have helped establish caffeine as an integral part of daily life around the world, serving cultural, religious, and medicinal purposes throughout history (Anderson, 2003; Weinberg & Bealer, 2001). As a result, caffeine's natural ubiquity and cultural value have made it exceedingly rare—if not impossible—to find individuals entirely unexposed to it (Nehlig, 2010). Consequently, daily caffeine consumption can be seen as a given and must therefore be taken into account when examining its effects on cognitive functions such as memory, particularly given that habitual intake can lead to tolerance and modulate its physiological impact (Lara et al., 2019).

Importantly, caffeine intake is typically assessed using dietary surveys, a method prone to underreporting due to missing details about the strength, brand, or quantity of consumed products. Participants also tend to only report common caffeine-containing beverages like coffee and tea, while overlooking other caffeine sources such as chocolate, over-the-counter analgesics, and appetite suppressants (Gracia-Lor et al., 2017; Nehlig et al., 1992). Compounding the issue, there is considerable variability in caffeine content across coffee products. For instance, significant differences have been observed between coffees of the same type from the same store (McCusker et al., 2003), across commercial coffee shops (Ludwig et al., 2014), between decaffeinated coffees from different shops (McCusker et al., 2006), among instant coffee brands, and even between cups prepared by different individuals using the same jar (Stavrić et al., 1988). These discrepancies likely stem from natural variations in caffeine levels between individual beans (Fox et al., 2013) and from differences in roasting temperature (Misto et al., 2021). As a result, such inconsistencies substantially reduce the accuracy of population wide and individual caffeine consumption estimates, especially considering that coffee is the primary source of caffeine in most countries (Fredholm et al., 1999).

In sum, the near-universal consumption of caffeine renders it both impractical and unnecessary to rely exclusively on caffeine-naïve participants in experimental research. Instead, daily caffeine consumption must be taken into consideration, particularly given the role of tolerance in modulating caffeine's cognitive effects. However, accurately quantifying both individual and national consumption rates remains problematic due to the limitations of self-reports and the inherent variability within and between caffeine-containing products. This issue

not only decreases the accuracy of tolerance estimates but also complicates the choice of experimentally administered doses. Therefore, although tolerance must be considered and national consumption rates can offer a ballpark estimate of what constitutes a moderate dose, both are likely only rough estimates that do not reflect true consumption patterns.

Psychoactive Effects of Caffeine

Caffeine and Lower Cognitive Functions

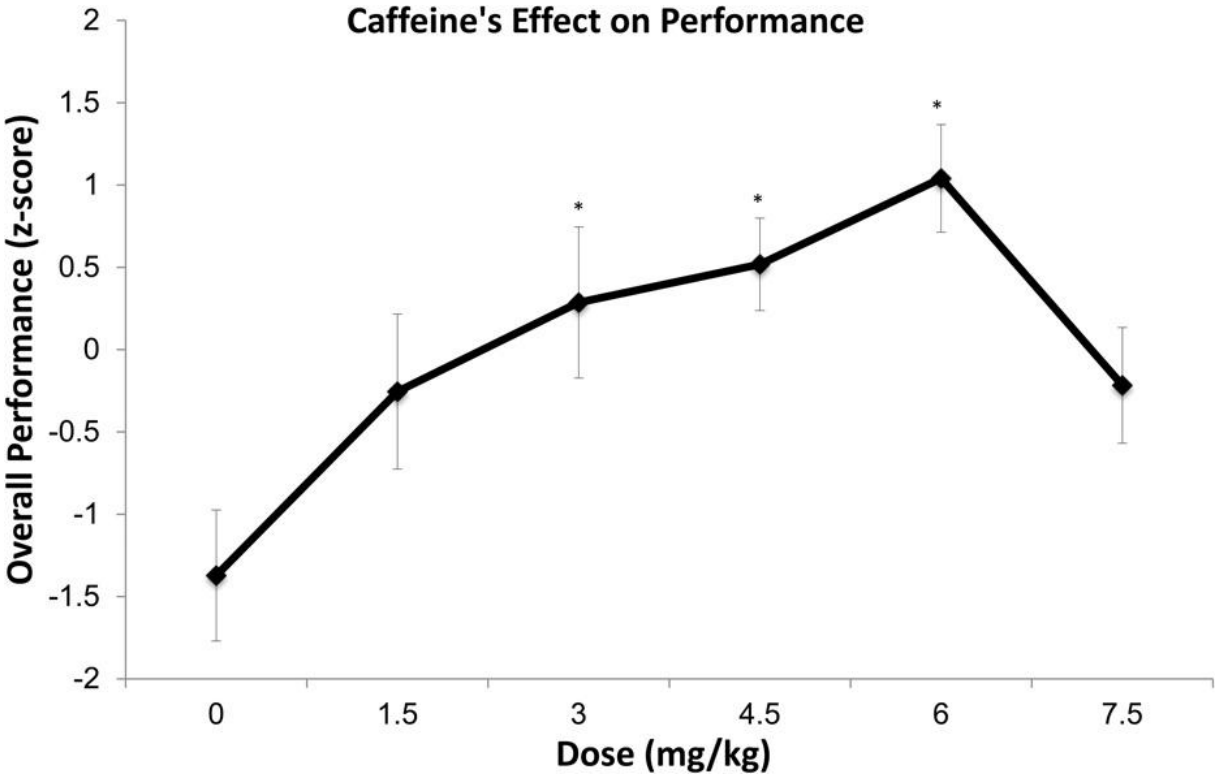
While caffeine's ubiquity and its historical significance have undoubtedly contributed to its widespread consumption, the compound's psychostimulant properties are certainly another considerable driving force behind its global popularity. Recognized for centuries for its stimulating properties and therapeutic applications, caffeine has traditionally served as a popular method for augmenting diverse aspects of cognitive and physical performance (Fredholm, 2011; Snel & Lorist, 2011). In contrast, academic interest in caffeine's stimulant effects on humans only emerged during the previous century when Hollingworth (1912) published the first placebo-controlled double-blind study on the effects of caffeine on human performance. Since then, a plethora of studies have investigated the effects of caffeine on cognitive and physical functions. Some of the more established psychoactive effects of caffeine include increased arousal (Doyle et al., 2016), enhanced vigilance and attention (Einöther & Giesbrecht, 2012), reduced reaction time (Cysneiros et al., 2007; Jacobson & Edgley, 1987; Santos et al., 2014), improved performance in endurance exercises (Southward et al., 2018), and positive effects on subjective mood (Childs & De Wit, 2006; Smit & Rogers, 2000).

Notably, these beneficial effects seem dependent on the administered dose, with empirical evidence suggesting an inverted U-shaped dose-response relation between caffeine and performance (see Figure 1). In other words, low to moderate doses are associated with enhanced performance, whereas performance tends to decline when doses exceed 300mg (Borota et al., 2014; Doyle et al., 2016; Kaplan et al., 1997; Lorist & Tops, 2003; Nehlig, 2010). Given its similar inverted U-shape and impact on arousal, caffeine's effect on performance has been linked to the Yerkes-Dodson law (see Figure 2), which states that increasing arousal leads to better performance but only up to a certain point (Yerkes & Dodson, 1908). Therefore, the initial arousal level of an individual before caffeine consumption can play a crucial role in determining the subsequent effects on performance, potentially explaining why caffeine's dose-response relation equally follows an inverted U-shape. For example, a fatigued individual's performance is likely to be enhanced by a large dose of caffeine, but giving the same dose to a

well-rested person may lead to excessive arousal, resulting in a worsened performance. This notion has led to a lack of consensus on the effects of caffeine, with some arguing that the positive effects are due to withdrawal reversal rather than actual performance enhancement (James & Rogers, 2005). However, studies with non-deprived and briefly deprived participants, as well as trials including multiple levels of habitual consumption, indicate that the positive effects of caffeine cannot be solely attributed to withdrawal reversal (Einöther & Giesbrecht, 2012). Overall, these findings suggest that caffeine typically has a net positive effect on a multitude of basic processes such as attention and vigilance, even though the level of arousal before consumption can considerably influence its effect.

Figure 1

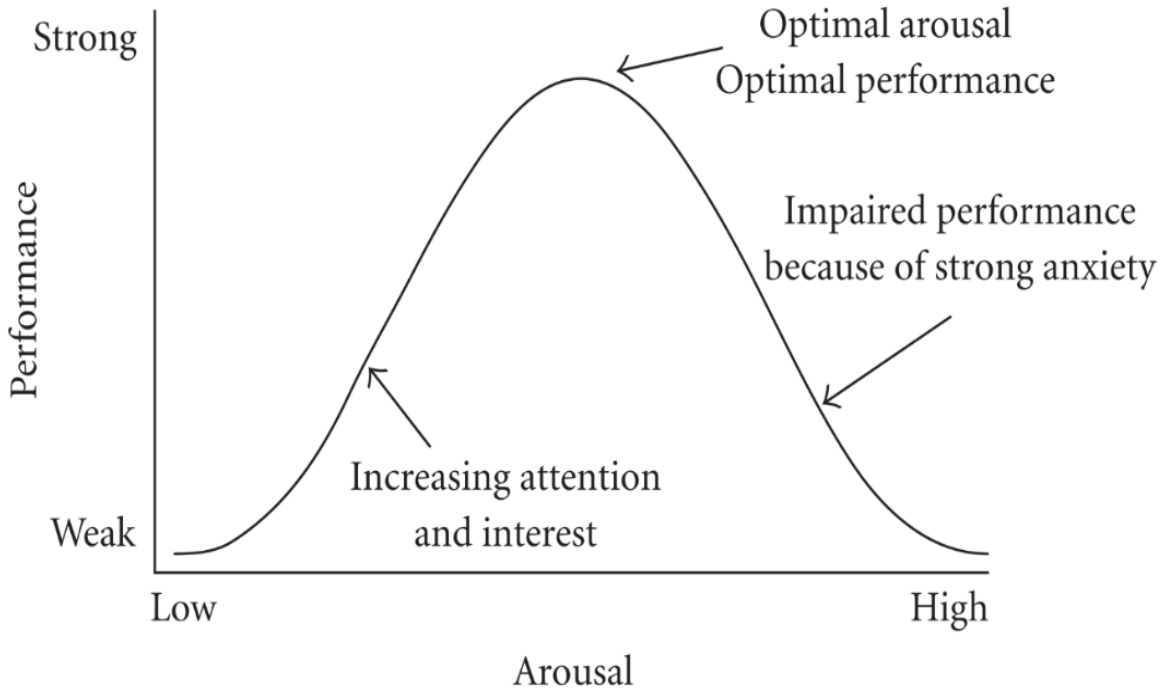
Typical Dose-Response Relation Between Caffeine and Performance



Note. Reprinted with permission from "The Effects of Caffeine on Arousal, Response Time, Accuracy, and Performance in Division I Collegiate Fencers," by P. T. Doyle, R. S. Lutz, J. K. Pellegrino, D. J. Sanders, S. M. Arent, *The Journal of Strength and Conditioning Research, 30*(11), p. 3233, (https://doi.org/10.1519/jsc.0000000000001602). Copyright 2016 by Wolters Kluwer Health, Inc.

Figure 2

The Yerkes-Dodson Law



Note. Reprinted from "The Temporal Dynamics Model of Emotional Memory Processing: A Synthesis on the Neurobiological Basis of Stress-Induced Amnesia, Flashbulb and Traumatic Memories, and the Yerkes-Dodson Law," by D. M. Diamond, A. M. Campbell, C. R. Park, J. Halonen, & P. R. Zoladz, 2007, *Neural Plasticity*, p. 1–33 (https://doi.org/10.1155/2007/60803). CC BY 3.0.

Caffeine and Memory

The relation between caffeine and memory is less well understood and considerably complex for multiple reasons. Firstly, caffeine typically improves lower cognitive functions (i.e., automatic, unconscious processes such as vigilance) whereas its effect on higher cognitive functions (i.e., voluntary, conscious processes such as problem solving) remains a matter of debate (Shallice, 1988; McLellan et al., 2016). However, declarative memory (i.e., consciously retrieved knowledge about facts and events) is generally considered a higher cognitive function, whereas non-declarative memory (i.e., unconsciously learned habits and skills in response to sensory input) is typically seen as a lower cognitive function (Sridhar et al., 2023). Therefore, the effects of caffeine on memory are likely specific to the type of memory assessed. Notably, the uncertainty regarding caffeine's effects on higher cognitive functions may be a consequence

of the limited number of published studies on the topic, along with significant variations in the methodologies used in the existing research (Brunyé et al., 2010). For instance, inconsistent findings regarding the effects of caffeine on memory can be attributed to variations in assessment methods (e.g., recall versus recognition) and the timing of assessments (e.g., immediate versus delayed; Nehlig, 2010).

Another point of complexity in the relation between caffeine and memory arises when differentiating between modes of learning. Caffeine can have a positive influence on verbal memory when information is absorbed passively (i.e., incidental learning) and recalled later. However, caffeine does not benefit memory when information is learned intentionally, suggesting that the relation between caffeine and memory is dependent on how new information is acquired (Nehlig, 2010).

Further, the impact of caffeine intake on declarative memory is influenced by an interaction between age and the time of day at which caffeine is consumed. For young adults, consuming caffeine in the morning leads to improvements in declarative memory whereas such enhancements are not observed when caffeine is consumed in the afternoon (Sherman et al., 2016). In contrast, older adults benefit from caffeine consumption in the afternoon, but not in the morning (Ryan et al., 2002).

Finally, many studies have concluded that caffeine has little to no effect on long-term memory retention. However, caffeine is typically administered before learning new information. When administered post-learning, caffeine has been shown to improve the retention of learned material (Angelucci et al., 2002; Borota et al., 2014). This improvement is thought to result from its effect on memory consolidation, a process by which memories become progressively stabilized after acquisition (Dudai, 2004). Therefore, it is likely that the effect of caffeine on long-term memory depends on the timing of consumption. Additionally, administering caffeine post-learning isolates the effect of caffeine to memory processes. If caffeine is consumed before or during a task, then changes in performance can also be attributed to increases in vigilance and attention instead of memory processes, thereby confounding effects of an intervention. Nevertheless, only Borota et al. (2014) report such results with human participants, raising doubts about the robustness of the effect.

Taken together, these findings suggest that caffeine administered after learning can improve procedural memory consolidation, especially since procedural memory is a lower cognitive function that is typically acquired through passive, incidental learning. However, it is unclear

whether this relation is equally subject to the interaction between age and time of day that has been observed with declarative memory.

Pharmacokinetics of Caffeine

Caffeine is known for a rapid and efficient absorption, with nearly 99% of the administered caffeine dose being absorbed from the stomach, the small intestine and the oral mucosa (Burdan, 2015). Consequently, increases in dosage result in proportionate, linear increases in caffeine levels in the blood plasma (Reddy et al., 2024). Moreover, the amount of caffeine absorbed remains unaffected by age, gender, genetic factors, health status, and the concurrent use of alcohol or nicotine. Typically, peak caffeine concentrations are reached in 30 to 60 minutes following oral ingestion (Arnaud, 2010). Nevertheless, there are notable disparities in the rate of absorption depending on the vehicle of administration. For instance, caffeine absorption from caffeine chewing gum is significantly faster in comparison to caffeine capsules (Kamimori et al., 2002). Similarly, caffeine from coffee and cola beverages is absorbed more quickly than from capsules, with both beverages reaching peak concentrations at similar rates (Liguori et al., 1997).

Once absorbed into the bloodstream, a small portion of caffeine molecules reversibly bind to plasma proteins, while the majority are rapidly distributed to various body tissues and organs. This distribution is facilitated by caffeine's hydrophilic nature, enabling it to easily enter intracellular tissue water. Additionally, caffeine's lipophilic properties allow it to cross biological membranes, including the blood-brain and placental barriers. As a result, caffeine is able to reach high concentrations in the brain, which enables its psychoactive effects (Reddy et al., 2024).

Caffeine metabolism (i.e., the speed at which caffeine is eliminated from the body) occurs primarily in the liver (Burdan, 2015) and exhibits considerable inter-individual variability. While the average elimination half-life of caffeine stands at approximately 3.7 hours, there are substantial differences among individuals, with caffeine half-lives ranging from 2 to 10 hours between people. Notably, caffeine metabolism is accelerated in individuals using nicotine and slowed down in women using oral contraceptives (Arnaud, 2010; Snel & Lorist, 2011).

In sum, despite individual differences in metabolism, experimentally administered caffeine is likely to exert broadly comparable effects across participants as its absorption is robust to individual differences. This is especially the case when caffeine is delivered in capsule form because capsules offer a consistent and controlled means of administration. In contrast,

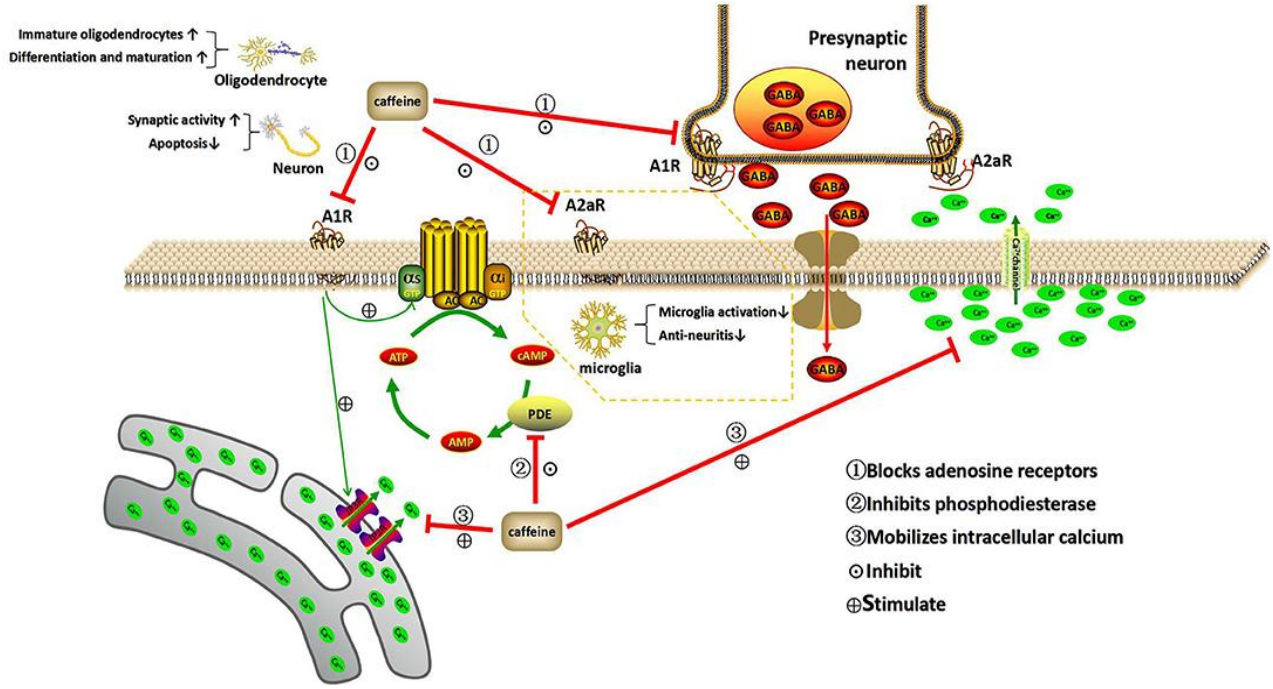
beverages such as coffee can contain additional bioactive compounds such as chlorogenic acid (Stefanello et al., 2019) and caffeine chewing gum dosage can be influenced by individual chewing habits (Banakar et al., 2022).

Mechanisms of Action

Caffeine predominantly exerts its effects by blocking adenosine receptors, which is the most significant mechanism at typical levels of caffeine consumption. Additionally, caffeine is known to inhibit the activity of phosphodiesterase and promote the release of stored intracellular calcium (see Figure 3), although these effects are typically only observed with very high doses (Reddy et al., 2024). As an inhibitory neuromodulator, adenosine influences both excitatory and inhibitory neurons in the central nervous system, with a more pronounced inhibitory effect on the release of excitatory neurotransmitters. This modulation occurs through the binding of adenosine to specific receptors in the brain, primarily adenosine A1 and A2a receptors (Porkka-Heiskanen et al., 2002). As an adenosine antagonist, caffeine diminishes the suppressive effects of adenosine by disrupting its receptors (Ferré, 2008; Fredholm, 1995; Ribeiro & Sebastião, 2010).

Figure 3

Caffeine's Main Mechanisms of Action



Note. Reprinted from "Encephalopathy in Preterm Infants: Advances in Neuroprotection with Caffeine," by Yang, L., Yu, X., Zhang, Y., Liu, N., Xue, X., & Fu, J. (2021), *Frontiers in Pediatrics*, *9* (https://doi.org/10.3389/fped.2021.724161). CC BY 4.0.

Importantly, these receptors exhibit different distribution patterns throughout the brain, which likely explain why caffeine exerts diverse effects. Adenosine A1 receptors are more widely spread in comparison to A2a receptors, with notably high levels in the hippocampus, cerebral cortex, and cerebellum. By binding to adenosine A1 receptors, caffeine blocks the natural inhibition of adenosine, resulting in increased neuronal activity in those regions. In turn, this increase in activity is believed to produce psychostimulant effects such as increased arousal, vigilance, and attention (Fisone et al., 2004). In contrast, adenosine A2a receptors are more concentrated in the striatum and are known to inhibit psychomotor function. By disrupting them, caffeine acts as a psychomotor activity enhancer (Ferré, 2010).

Although caffeine primarily affects adenosine, it also interacts indirectly with other neurotransmitters. To be specific, caffeine affects the dopaminergic system due to interactions between adenosine and dopamine receptors (Ferré et al., 1997; Fisone et al., 2004). Similarly, caffeine indirectly affects the release of noradrenaline, acetylcholine, serotonin, glutamate, and GABA. These indirect effects are often seen as another explanation for caffeine's diverse psychoactive effects (Ferré, 2010; Nehlig et al., 1992)

Since procedural memory is mainly associated with the striatum (Squire, 2004), it is likely that caffeine's effect on procedural memory could be predominantly driven by the antagonism of striatal adenosine A2a receptors having an indirect effect on dopamine and glutamate levels. More specifically, caffeine counteracts the inhibitory control exerted by adenosine A2a receptors, thereby increasing transmission of dopamine and glutamate (Fisone et al., 2004). In turn, an increased transmission of striatal dopamine (De Vries et al., 2010; Rammsayer et al., 2000) and glutamate (Gais et al., 2008; Kelley et al., 2003) can be beneficial for procedural learning.

Nonetheless, A1 receptors might equally play a key role due to their high density in the hippocampus and cerebellum (Fisone et al., 2004). Although the famous case of patient H. M. (Corkin, 1968) and multiple other amnesic patients (see Reber & Squire, 1994) previously suggested that the hippocampus is not required for procedural learning, recent findings have started to challenge this notion, indicating that the hippocampus is involved in both declarative and procedural learning. More specifically, the hippocampus considerably contributes to the

consolidation of procedural memory, even when it is not involved during the acquisition of non-declarative information (Albouy et al., 2013; Schapiro et al., 2019). Given that hippocampal dopamine (Jay, 2003; Neves et al., 2020; Titulaer et al., 2021), glutamate (Stanley et al., 2017; Thielen et al., 2018), and acetylcholine (Hasselmo, 2006) are all associated with declarative memory formation and consolidation, it is possible that caffeine might also influence hippocampal consolidation of procedural memories by modulating the release of these neurotransmitters. Similarly, cerebellar dopamine (Caragea et al., 2024), glutamate (Van Der Heijden et al., 2023), and acetylcholine (Prestori et al., 2013) are crucial for procedural learning, suggesting that caffeine's inhibitory effect on adenosine A1 receptors in the cerebellum might also contribute to procedural memory consolidation.

In sum, the potential effects of caffeine on procedural memory are likely mainly driven by its antagonising effect on adenosine A2a receptors in the striatum, although A1 receptors in the hippocampus and cerebellum might also play an important role in enhancing consolidation.

Importantly, memory consolidation involves two processes, namely synaptic consolidation (i.e., the stabilizing of newly formed memories at the level of synapses) and systems consolidation (i.e., the reorganization of information to the neocortex). Although both processes begin immediately after learning, each concludes at different timepoints. Typically, synaptic consolidation can range from minutes to hours while systems consolidation can take from days to weeks or more (Dudai, 2004). Therefore, caffeine probably affects the full course of synaptic consolidation and only partially affects systems consolidation when consumed immediately after learning, due to its average half-life of approximately 3.7 hours (Arnaud, 2010).

Despite the aforementioned reasons to believe caffeine might benefit procedural memory, no such investigations have been conducted. The current study addresses this gap by testing whether 80mg and 240mg of caffeine, corresponding to low and high daily doses in Belgium, can positively impact procedural memory consolidation when ingested post-learning. While caffeine's effects on performance typically follow an inverted U-shaped dose-response, we hypothesized a more linear improvement in procedural learning with increasing dosage, given that consolidation processes likely depend more on neurotransmitter modulation than on arousal levels.

Methods

Preregistration

This study's target sample size, main variables, hypotheses, and planned analyses were preregistered on Open Science Framework (osf.io/5dkpr). The dataset, analysis script, and stimuli presentation code can also be found on this project's Open Science Framework webpage.

Participants

Thirty participants, (*M^age* = 26, *SD* = 7.12, 57% male) were recruited for this study through flyers and word of mouth. An a priori power analysis using lem4 (Bates et al., 2015) and simr (Green & MacLeod, 2015) indicated that a sample size of 30 was sufficient to achieve a power of 90%. This calculation was made for a linear mixed model investigating a three-way interaction (Group x Day x Sequence), assuming an alpha level of .05, and small and moderate effects (Cohen's *d* = 0.2 and 0.5) between the active dose groups and the placebo group. A post hoc power analysis on the actual data set, with artificially injected small and moderate effects between the active dose groups and the placebo group, yielded a power of 71%.

Participants were instructed to abstain from all sources of caffeine, including caffeine-containing over-the-counter medications (e.g., Excedrin, Anacin), throughout the experiment. Participants were informed of the risks, potential benefits, and purposes of the study before written consent was obtained. Participants were not informed of the placebo condition before the end of the experiment to avoid expectation effects. In return for their participation, participants were entered into a raffle for a chance to win a €25 online shop voucher. The following groups were excluded from the study as there is some evidence that even low doses of caffeine can be harmful to certain individuals: pregnant women, individuals under 18 years of age, individuals with pre-existing heart conditions, patients with mental or psychiatric illnesses, and individuals taking lithium, clozapine, theophylline, fluvoxamine, mexiletine, psoralens, furafylline or enoxacin (Carrillo & Benitez, 2000; Temple et al., 2017). This study was approved by the Social and Societal Ethics Committee at the Katholieke Universiteit Leuven.

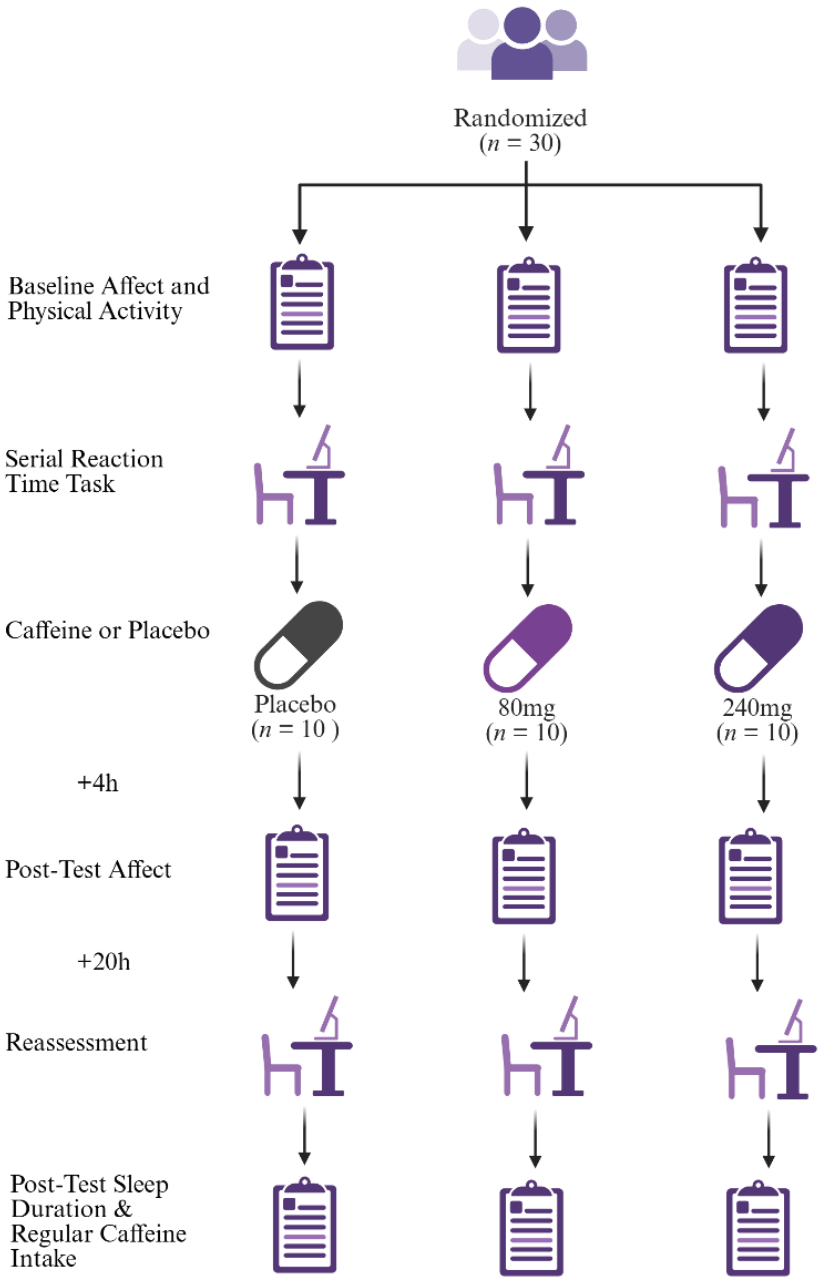
Procedure

This study used a double-blind, placebo-controlled, mixed design with two measurement moments. One individual that was not involved in any other aspect of the study was responsible for the random assignment of participants to a placebo (n = 10), low dose (80mg, n = 10), or high dose group (240mg, n = 10). Before the experiment, participants completed either the English or Dutch short version of the International Physical Activity Questionnaire (Craig et al., 2003), as physical activity has been shown to influence memory function and consolidation (Hopkins et al., 2012; Loprinzi et al., 2021; Schimidt et al., 2016; Thomas et al., 2016). Physical activity scores were computed following the protocol by Sjöström et al. (2005), reflecting weekly metabolic energy expenditure in minutes (Jetté et al., 1990). Participants also completed either the English (Thompson, 2007) or Dutch (Engelen et al., 2006) short version of the Positive and Negative Affect Schedule (PANAS) to assess their baseline subjective affect since emotional arousal is known to influence memory consolidation (LaLumiere et al., 2017; Lee & Sternthal, 1999). At the first measurement moment, participants completed the Serial Reaction Time Task (SRTT) after which they were instructed to ingest caffeine capsules (Pure Cafeïne®, Solidpharma, Belgium). Post-test PANAS scores were also collected four hours following capsule ingestion to assess caffeine-induced affect changes, given caffeine's documented positive effects on affect (Childs & De Wit, 2006; Smit & Rogers, 2000).

The second measurement moment took place 24 hours after the first, with participants returning to the testing location to complete the SRTT for a second time. A 24-hour interval between test sessions was employed to ensure full caffeine elimination. After completing the task, participants reported how many hours they had slept during the night after the first measurement, as sleep duration is a critical factor in procedural memory consolidation (Schönauer et al., 2014; Van Schalkwijk et al., 2017). Moreover, high doses of caffeine can disrupt sleep even when taken early in the day (Gardiner et al., 2023), which could influence consolidation outcomes. Participants were asked whether they noticed and could replicate the sequence of stimuli, to assess the potential contribution of declarative learning. Those who could fully reproduce the sequence were excluded from the analysis as this would likely reflect a reliance on declarative rather than procedural memory. Further, participants were also asked to guess whether they received caffeine or a placebo to evaluate the success of blinding. Finally, participants reported how many caffeinated beverages they typically consume per day as a measure of tolerance to caffeine. Figure 4 offers a visual illustration of the entire procedure.

Figure 4

Visual Illustration of the Experimental Design



Note. Created with BioRender.com.

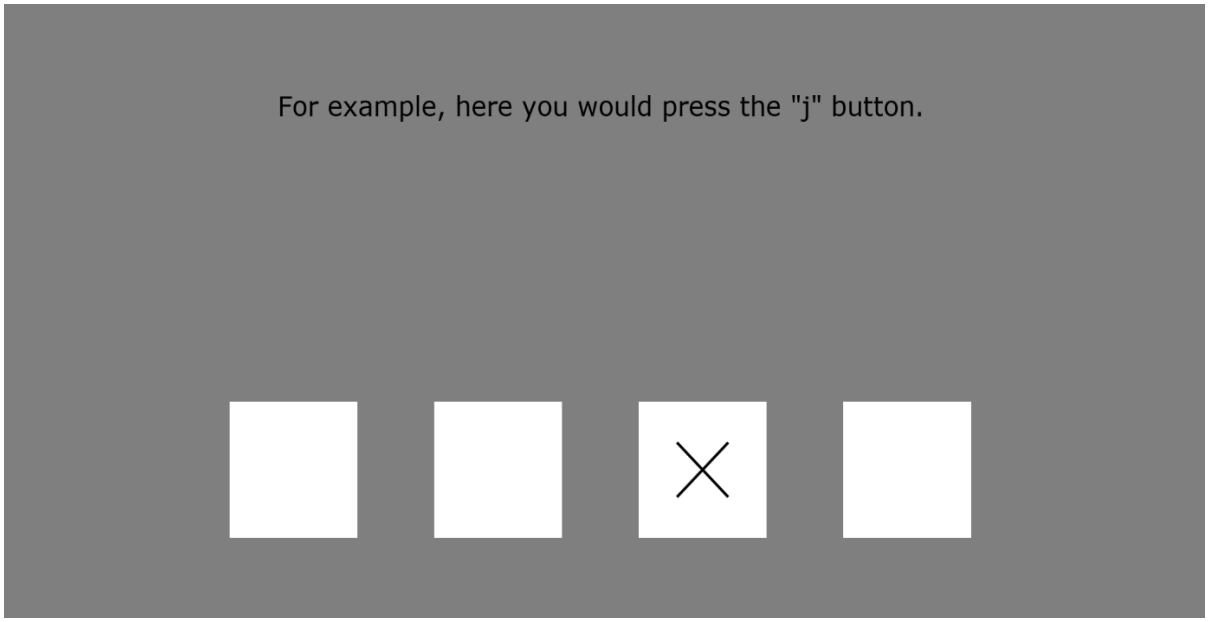
Learning Task

Participants completed the SRTT twice to assess procedural learning. During the task, participants were seated in front of a computer screen and were instructed to respond as quickly and accurately as possible to visual stimuli by pressing a corresponding key on a keyboard. The stimuli consisted of four horizontally aligned white squares displayed on a grey background,

each corresponding to a specific key on the keyboard (i.e., from left to right: ‘S’, ‘D’, ‘J’, and ‘K’). During each trial, a black cross appeared in one of the squares for two seconds, and participants were instructed to press the corresponding key. For example, if a cross appeared in the first square counting from left to right, participants had to press the ‘S’ key, but if it appeared in the third square, they were expected to press the ‘J’ key (see Figure 5). Unbeknownst to the participants, the crosses were presented following two different 12-item second-order probabilistic sequences (i.e., sequences where the probability of the next item depends on the two preceding items in the sequence). The crosses were presented following the probable sequence A (SDSKJDKSJKDJ), presented with a probability of .85, or according to the improbable sequence B (JDJKSDKJSKDS), presented with a probability of .15. A second-order probabilistic SRTT was preferred over the classic deterministic version because it offers a greater resistance to explicit awareness of the sequence compared to deterministic versions (Schwarb & Schumacher, 2012).

Figure 5

SRTT Trial Shown During the Practice Phase



The full task comprised of two phases, a practice phase of 20 trials and a testing phase of 10 blocks of 40 trials. Although a total of 25 blocks of 85 trials is recommended to reach minimally acceptable levels of reliability (Farkas et al., 2024), task length was limited to prevent fatigue and attentional lapses, which could undermine data quality. The practice phase served to familiarize the participants with the task and could be repeated indefinitely. Reaction

times (RT) and key presses were recorded for each trial of the testing phase, with RT being defined as the time in seconds elapsed from the appearance of the cross to the participant's key press. Pressing any of the specified keys ended a trial and prompted the next trial, but pressing unrelated keys (e.g., the 'Z' key) had no effect. Pressing the wrong specified key (e.g., pressing the 'K' key when the cross was in the first square) or failing to react within two seconds was counted as a mistake and elicited a beeping noise to alert the participants of the wrong input. Participants could take breaks between each block of the testing phase for up to 30 seconds.

The main goal of the task is to identify differences in RT and accuracy between the probable and improbable trials as this provides an indication that participants have learned the sequence and are therefore able to implicitly predict the next position of the stimuli. In this regard, lower RT and higher accuracy on the probable sequence in comparison to the improbable sequence reflect that procedural learning has taken place (Schwarb & Schumacher, 2012). Finally, since the SRTT was measured twice in this study, differences in RT between measurement moments reflected the offline consolidation of the learned sequence between days.

Materials

All caffeine capsules were obtained from Solidpharma, Belgium, while placebo capsules were produced in-lab. All capsules were odourless and similar in colour, taste and texture. The SRTT presentation and data logging code was written in MATLAB (Natick, 2024) and relied on the Psychophysics Toolbox extensions (Brainard, 1997; Pelli, 1997; Kleiner et al, 2007) for stimulus presentation. The code was an adaptation of the publicly available SRTT by Parsons (2015) and can be found on OSF.

Hypotheses

Given that caffeine has the potential to enhance procedural memory consolidation, we expected that on day two, the low dose and the high dose group would exhibit lower RT on the probable sequence in comparison to the placebo group. Further, this effect would be subject to a dose-response relation whereby the high dose group would show a larger reduction in RT on the probable sequence in comparison to the low dose group. In other words, we expected an interaction effect on RT between the sequence, day, and group (H1). Moreover, enhanced learning due to caffeine might also be reflected in more mistakes on the improbable sequence

due to a developed rigid automaticity to the probable sequence. Therefore, we expected an interaction effect on accuracy between the sequence, day, and group (H2).

Analyses

All analyses were conducted using R (R Core Team, 2024) within RStudio (Posit Team, 2025). Missing values were imputed through predictive mean matching (Little, 1988; Rubin, 1986) using the mice package (Van Buuren & Groothuis-Oudshoorn, 2011). All visualizations were generated with the ggplot2 package (Wickham, 2016) while data frame manipulations relied on the dplyr library (Wickham et al., 2023).

Group Comparisons on Control Variables

Post-hoc analyses were conducted on baseline control variables to confirm the success of random assignment. This included group comparisons in baseline subjective affect, baseline physical activity, and regular caffeine intake. These analyses were not hypothesis-driven but served to verify group equivalence prior to the intervention. No significant differences were observed across groups for any baseline variable, suggesting successful randomization (see Figure A1 and Table A1 in Appendix A). Further, a chi-square test of independence was conducted to examine whether participants' beliefs about their group assignment corresponded to their actual treatment condition. The association between actual group and correct guess was not statistically significant, $\chi^2(2)$, $n = 30) = 0.80$, $p = .67$, suggesting successful blinding across conditions.

Psychometric Properties of the SRTT

Test-retest reliability and split-half reliability were computed to assess the psychometric properties of the SRTT. Test-retest reliability was measured by calculating the Pearson correlation between RT on the first day and RT on the second day. This was computed once on the entire dataset and once per group because offline consolidation between measurement moments typically decreases test-retest reliability (Farkas et al., 202) and the groups could differ in terms of consolidation processes due to the intervention. Split-half reliability was calculated by splitting the dataset into odd and even halves and then computing the Spearman-Brown correlation between halves. The Spearman-Brown formula was chosen because it estimates the reliability of an entire set from the reliability of its two halves (Kingston & Tiemann, 2010). Test-retest reliability was above the acceptable psychometric standard of $r >$

.70 (Nunnally & Bernstein, 1994, as cited in Oliveira et al., 2024) and differed slightly between groups. Split-half reliability was slightly below the psychometric standard but within the range of previously reported SRTT split-half reliability estimates (Oliveira et al., 2024). Reliability scores are presented in Figure B1 and Table B1 within Appendix B.

Mean RT and accuracy per block were plotted to investigate whether fatigue affected performance throughout the task. This was formally confirmed by computing generalized linear mixed-effects model (GLMM) predicting trials-level RT and mistakes with block number as the sole predictor. Fatigue did not influence RT nor accuracy (see Figure B2 in Appendix B).

The Effect of Caffeine on RT

To test whether caffeine influenced RT (H1), a linear mixed effects model (LMM) was initially computed, as preregistered. Due to positive skew in the RT distribution, the response variable was log-transformed to approximate normality. The model thus predicted log-transformed RT by the sequence, testing day, group, and their interactions, along with baseline positive affect, negative affect, and physical activity as covariates. Interactions between the testing day and post-test variables, namely positive affect, negative affect, sleep duration, and regular caffeine intake, were included to capture their influence on RTlog solely on day two. To achieve this, testing day was dummy-coded and the main effects of these post-test variables were omitted. All continuous covariates were *z*-standardized prior to model estimation to improve interpretability and model convergence. Random intercepts and random slopes for the testing day and sequence were included to account for individual differences in baseline RTlog and learning trajectories.

Visual model diagnostics indicated that assumptions of linearity and homoscedasticity were met, with no evidence of curvature or heteroscedastic spread in the residuals. However, the residual Q-Q plot showed a strong deviation at the tails and the presence of outliers, introduced after log transform, suggesting a violation of normality (see Figure C1 in Appendix C). Additionally, bootstrapped regression lines, which do not rely on parametric assumptions, indicated a clearer group-level effect than what was captured by the LMM, suggesting that the model's estimates were influenced by the violation of these assumptions (see Figure C2 in Appendix C).

To address these concerns, the preregistered model was discarded, and a GLMM was estimated using an inverse Gaussian distribution with an identity link. This model was theoretically more appropriate for RT data in comparison to a LMM with log transformed RT

(Lo & Andrews, 2015) and included the same fixed and random effects structure as the LMM. The assumptions of this model were met as RT followed an inverse Gaussian distribution, a linear relation between the function link and the mean of RT could be established, and multicollinearity did not pose a significant issue (see Figure C3 and Table C1 in Appendix C).

Model fit was evaluated by comparing conditional R^2, Root Mean Squared Error (RMSE), and Akaike's Information Criterion (AIC; Akaike, 1974) between nested models. Covariates were excluded if their removal led to a reduction in AIC of two or more (see Burnham & Anderson, 2004), or if their Variance Inflation Factor (VIF) exceeded ten (see Hair et al., 2005). Dummy-coded categorical variables were exempt from the VIF criterion since high multicollinearity is expected with such variables, especially in models with interaction terms, and generally does not pose significant issues (Allison, 2012). Performance indicators for all model comparisons are presented in Table C2 in Appendix C.

The Effect of Caffeine on Accuracy

To investigate the effect of caffeine on accuracy (H2), GLMM with a binomial log-link function was fitted to predict trial-level accuracy operationalized as a binary variable (Mistake = 1, Correct = 0), as preregistered. The fixed effects structure included the groups, testing day, sequence, and their interactions, along with baseline positive, negative affect and physical activity level. Interactions between the testing day and post-test positive affect, negative affect, sleep duration, and regular caffeine intake were included, with testing day dummy-coded so that these variables only contributed to the model on day two trials. Random intercepts and slopes for the testing day and sequence were included to account for individual differences in baseline accuracy. All numeric covariates were *z*-standardized prior to model estimation.

This model and its nested variants all failed to converge. To address this, accuracy was aggregated at the block level (20 blocks per participant, across both days and sequences), and the outcome variable was transformed to proportional accuracy per block. A GLMM with a beta distribution and logit link function was then fit to the simplified dataset. However, this model was also discarded due to severe violations of distributional assumptions, as indicated by simulation-based residual diagnostics. Consequently, a non-parametric aligned rank transform ANOVA (Wobbrock et al., 2011) was conducted using the ARTool package (Elkin et al., 2021) to evaluate the interaction between group, testing day, and sequence on proportional accuracy. This model included random slopes for testing day within participants,

but omitted covariates due to methodological constraints. Post-hoc pairwise comparisons were conducted using a linear model on the aligned response values.

Use of Generative Artificial Intelligence

ChatGPT (OpenAI, 2023) was utilized to generate R and MATLAB code suggestions and to support manuscript revisions aimed at improving conciseness, clarity, flow, and grammar. All suggestions were critically evaluated and extensively modified prior to incorporation. ChatGPT was not employed as a primary source of information nor for summarizing external materials.

Results

Effects on RT

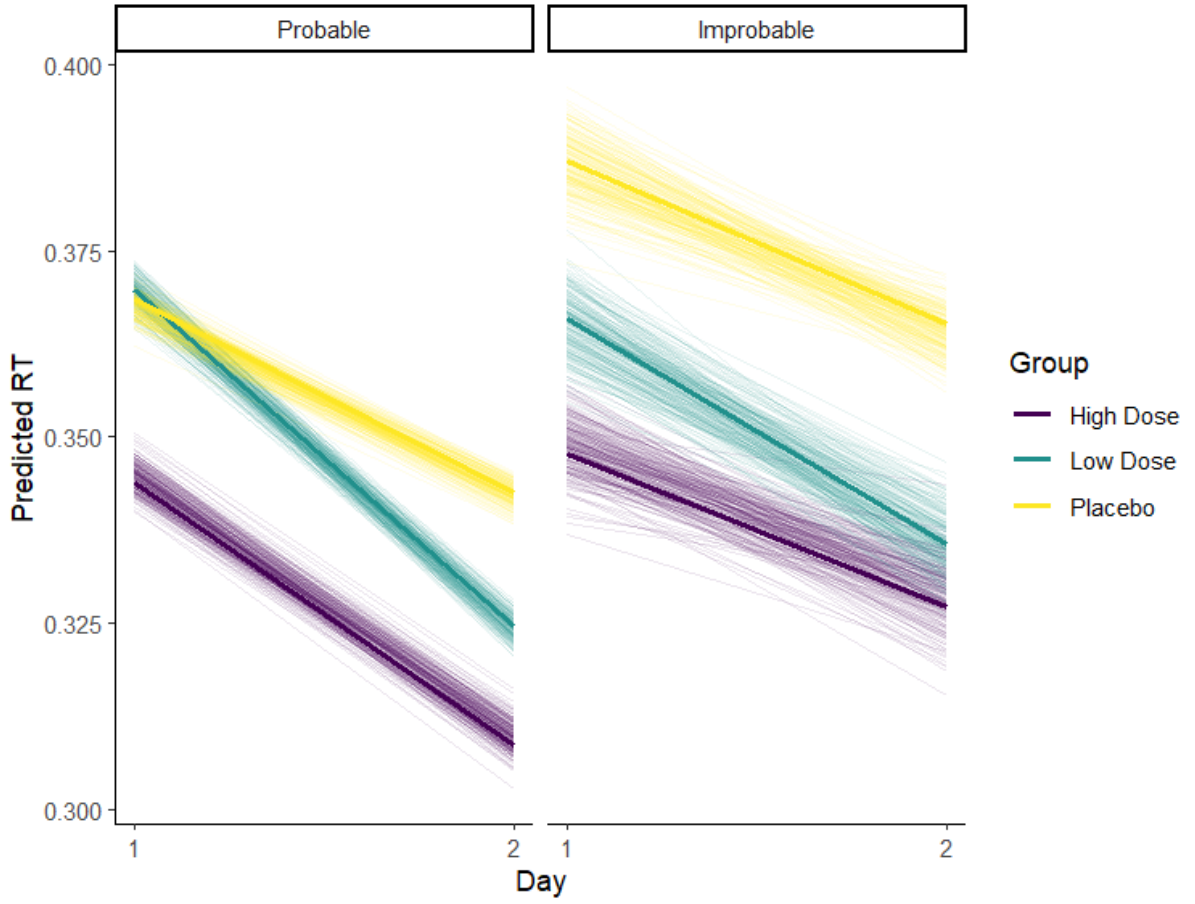
A GLMM was fitted to predict RT based on task sequence, testing day, treatment group, their interactions, and relevant covariates (see Methods). Participants in the placebo group demonstrated significantly faster performance on day two compared to day one, reflected in a negative main effect of testing day, $\beta = -0.026$, $p = .005$, corresponding to a 26ms reduction in RT between days. The sequence significantly predicted RT, $\beta = 0.019$, $p < .001$, such that participants in the placebo group were 19ms slower on the improbable sequence compared to the probable sequence on day one, as expected. Baseline positive affect was also a significant predictor, $\beta = -0.039$, $p < .001$, indicating that for each standard deviation increase in baseline positive affect, RT decreased by approximately 39ms.

A significant interaction between the sequence and the low dose group was observed, $\beta = -0.023$, $p = .017$, reflecting an approximate 23ms reduction in RT on the improbable sequence in the low dose group relative to the placebo group on day one. This interaction was not significant in the high dose group, $\beta = -0.015$, $p = .112$. Moreover, a significant interaction between testing day and the low dose group emerged, $\beta = -0.019$, $p = .041$, indicating that 80mg of caffeine ingested post-learning was associated with an additional 19ms decrease in RT between days compared to placebo. Though a visualization of the model's predictions with bootstrapped regression lines hinted at a similar interaction between testing day and the high dose group (see Figure 6), no such effect was observed in the 240mg group, $\beta = -0.010$, $p = .353$. Further, a significant interaction was observed between testing day and self-reported regular caffeine intake, $\beta = 0.010$, $p = .024$, suggesting that greater habitual caffeine use was associated with a 10ms increase in RT on day two. The hypothesized three-way interactions

between sequence, testing day, and group did not reach statistical significance in the low dose group, β = 0.011, *p* = .161, nor the high dose group, β = 0.011, *p* = .166.

Figure 6

Model-Predicted RT with Bootstrapped Regression Lines per Sequence



Neither baseline negative affect, β = −0.008, *p* = .383, nor baseline physical activity, β = −0.005, *p* = .595, significantly predicted RT. Similarly, group membership alone did not significantly predict RT for either the low dose group, β = 0.001, *p* = .955, or high dose group, β = −0.025, *p* = .332. No other two-way interactions reached significance, including the sequence by testing day interaction, β = 0.004, *p* = .484, and the post-test sleep duration by testing day interaction, β = 0.004, *p* = .425.

Three-way interactions between group, testing day, and post-test positive affect were not significant in the placebo group, β = 0.010, *p* = .214, the low dose group, β = 0.004, *p* = .534, or the high dose group, β = 0.005, *p* = .432. Similarly, three-way interactions between group, testing day, and post-test negative affect were not significant in the placebo group, β = −0.014,

p = .075, the low dose group, β = −0.007, p = .303, or the high dose group, β = −0.004, p = .487.

A negative correlation was observed between the individual intercepts and day slopes, r = -.49, indicating that faster participants tended to improve less across days. Similarly, a negative correlation was found between the intercept and sequence, r = -.25, suggesting that faster participants were less affected by the improbable sequence. Moreover, those who improved more across testing days were slightly more affected by sequence differences, as evidenced by a positive correlation between testing day and sequence slopes, r = .20. Faster participants also exhibited a larger sensitivity to sequence differences across days as the correlation between the intercepts and the day x sequence interaction was positive, r = .29. Finally, the correlation between the individual slopes and the day x sequence interaction was negative, r = -.23, indicating that those with a strong sequence effect showed a weaker sequence effect across days.

Effects on Accuracy

A non-parametric aligned rank transform ANOVA was conducted to assess the effects of sequence, testing day, and group on accuracy, operationalized as the proportion of correct responses (see Methods). The analysis revealed a significant main effect of sequence, F(1, 1134) = 55.66, p < .001, with participants performing approximately 1.8% more accurately on the probable sequence (M = 96.9%) than on the improbable one (M = 95.1%). A significant interaction between group and testing day emerged, F(2, 27) = 7.70, p = .002, indicating that accuracy changes across days varied by group. More specifically, the placebo group declined by 1.1% (97.3% to 96.2%), the low dose group decreased by a smaller margin of 0.4% (96.9% to 96.5%), while the high dose group improved by 0.5% (96.3% to 96.8%). Additionally, a significant group-by-sequence interaction was observed, F(2, 1134) = 4.11, p = .017. Accuracy dropped by 2.6% on the improbable sequence in the placebo group (97.1% to 94.6%) and by 2.5% in the high dose group (96.9% to 94.5%), whereas the low dose group exhibited a smaller decline of 0.6% between sequences (96.8% to 96.2%).

The hypothesized three-way interaction among sequence, testing day, and group was not significant, F(2, 1134) = 1.78, p = .170. Neither the main effects of testing day, F(1, 27) = 0.17, p = .685, nor group, F(2, 27) = 1.53, p = .235, were significant. The day by sequence interaction also did not reach significance, F(1, 1134) = 0.20, p = .655. Post-hoc comparisons using

estimated marginal means based on aligned response values did not yield any significant pairwise differences after correction for multiple comparisons (all *p*-values > .81).

Discussion

Post-Learning Caffeine Enhances Procedural Memory Consolidation

The present study investigated whether post-learning caffeine ingestion enhances procedural memory consolidation, measured by sequence-specific changes in RT and accuracy between consecutive days. While the hypothesized three-way interaction between sequence, testing day, and group was not observed, key significant two-way interactions provided support for the notion that caffeine intake can facilitate procedural memory consolidation.

Participants who received a low dose containing 80mg of caffeine exhibited faster RT on the improbable sequence, compared to placebo on day one. However, a similar interaction was not found for participants who received a 240mg dose of caffeine. Importantly, participants in the 80mg group also showed a greater improvement in RT between sessions compared to placebo, but the same was not seen in the 240mg group. These findings suggest caffeine intake may support consolidation, reflected in RT changes, in a dose dependent manner.

Furthermore, higher regular caffeine consumption was associated with slower RT on day two, suggesting that habitual caffeine use may attenuate any potential benefits of acute intake. Baseline positive affect also predicted faster RT, while post-test positive affect did not, indicating that the beneficial effect of caffeine was likely not mediated by mood enhancement. Notably, these findings imply that pre-test positive mood can enhance the acquisition of procedural information, possibly by boosting attentional resources (see Vanlessen et al., 2016), though whether this translates to better sequence acquisition or simply increased response readiness remains uncertain.

Regarding accuracy, participants generally performed better on the probable sequence, confirming that the SRTT elicited the expected learning effects. However, no significant three-way interaction emerged for accuracy either. Nevertheless, the low dose group demonstrated more stable accuracy across days and sequences compared to the other groups, which contradicts the hypothesized decrease in accuracy due to enhanced consolidation. On the contrary, these results suggest a low dose of caffeine post-learning may promote consistency in performance over time.

Taken together, these findings indicate that moderate caffeine intake following learning may improve procedural memory consolidation both in terms of RT and accuracy. More

specifically, post-learning caffeine intake may improve overall task efficiency given that participants who received caffeine doses were both faster and exhibited more stable accuracy in comparison to participants who received a placebo or a high dose.

Interestingly, the effect of caffeine dosage varied depending on the performance metric. While accuracy improved linearly in a dose-dependent manner, RT followed an inverted U-shaped pattern, with 80mg yielding the strongest benefit. The latter mirrors well-documented findings in the caffeine literature (see Borota et al., 2014; Doyle et al., 2016; Nehlig, 2010) and may reflect arousal-related mechanisms consistent with the Yerkes-Dodson law (Yerkes & Dodson, 1908). However, it is unclear why this pattern does not extend to accuracy. Further, measures of post-test affect, both positive and negative, did not significantly predict performance, challenging the interpretation that consolidation enhancements in RT follow an inverted U-shape due to changes in arousal. Therefore, it is likely that caffeine's dose-response relation to procedural memory consolidation is dependent on the aspect of performance that is assessed and cannot be fully explained by arousal modulation.

Finally, the practical significance of the effects should be interpreted with caution. While statistically significant, the magnitude of the performance gains from caffeine was relatively modest, especially compared to effects associated with baseline positive affect. Furthermore, the known sleep-disrupting effects of caffeine, particularly when consumed within 8–13 hours of bedtime (Gardiner et al., 2023), could counteract any benefits for consolidation since sleep is an established, crucial factor in procedural memory consolidation (Schönauer et al., 2014; Van Schalkwijk et al., 2017). Additionally, regular caffeine intake use was related to decreased consolidation, suggesting diminishing returns with frequent consumption. Taken together, these findings suggest that avoiding caffeine intake might be more beneficial to procedural memory consolidation when taking the magnitude of its effect and the risk of disrupting sleep into account. Consequently, these findings raise the possibility that caffeine may not be the most efficient route to enhancing procedural memory consolidation. Instead, interventions targeting positive affect, if shown to enhance acquisition rather than just attention, could provide a more sustainable and less physiologically disruptive alternative.

Limitations

While these findings offer preliminary support for a role of moderate caffeine intake in enhancing procedural memory consolidation, several limitations constrain their interpretation and generalizability.

Sample homogeneity is a key issue. The study population consisted mostly of young psychology students, limiting the applicability of these results to other age groups or clinical populations. Given that caffeine's effects on declarative memory have been shown to vary by age and time of day (Ryan et al., 2002; Sherman et al., 2016), it is plausible that procedural memory might exhibit a similar moderation. Older adults, in particular, represent a crucial group for future research due to the prevalence of procedural memory deficits in conditions such as Parkinson's and Huntington's disease (Allain et al., 1995; Bylsma, 1990; Cayzac et al., 2011; Knopman & Nissen, 1991; Pauly et al., 2022). Moreover, since average caffeine consumption varies considerably between countries (Fredholm et al., 1999), it is uncertain to what extent these results apply to different regions, especially in light of the diminished beneficial effect observed in participants with higher habitual caffeine intake and the lack of a significant RT change in the high dose group. As a result, individuals from regions with lower average consumption may have a lower optimal physiological threshold to caffeine's effects, potentially rendering both doses in this study supra-optimal. Conversely, in populations with higher habitual intake, the lower dose may be insufficient to elicit measurable benefits, while only the higher dose might approach their effective threshold.

Similarly, the scope of procedural learning assessed in this study was relatively narrow. Although the SRTT is a well-established paradigm, it captures only a limited range of motor and cognitive procedural skills. Therefore, it remains unclear whether caffeine would have similar effects in more complex, real-world tasks such as musical skill acquisition, athletic training, or rehabilitation exercises. Moreover, two sessions might have been insufficient to reach a learning ceiling, making it difficult to conclude whether caffeine accelerated how quickly participants reached task mastery or whether it extended the natural limits of learning. If caffeine primarily accelerates mastery, strategically administered caffeine might benefit learners who are acquiring new skills, but offer minimal advantages to experts who have already achieved high proficiency. Additionally, whether repeated caffeine intake offers a consistent benefit to procedural memory consolidation remains an open question, although the current findings hint at diminishing improvements. Consequently, future research should include real-world procedural tasks, multiple training sessions, and participants varying in skill levels, to clearly delineate under what conditions caffeine enhances procedural memory consolidation.

Adherence to experimental instructions may also have introduced unwanted variability. For instance, participants were instructed to abstain from caffeine outside of the administered dose and to complete mood measures at specific times, but compliance was not verified. Any

unreported caffeine consumption could have confounded group assignments, especially if unevenly distributed. Similarly, variability in the timing of the post-test affect measurements might have missed caffeine-induced changes in mood if participants delayed PANAS completion beyond the intended timeframe.

On a fundamental level, the mechanistic explanation for caffeine's beneficial effects remains speculative. Although previous work has highlighted caffeine's indirect effect on the release of various excitatory neurotransmitters in the hippocampus, striatum, and cerebellum (Ferré et al., 1997; Fisone et al., 2004), which could influence consolidation processes, no physiological measures were included in this study. Thus, the functional and structural neural substrates underlying the observed improvements in consolidation remain uncertain. Nevertheless, past imaging work has revealed that motor skill consolidation is associated with decreased primary motor cortex activity (Fischer et al., 2005) and increased striatal activity (Debas et al., 2010). Therefore, caffeine's excitatory effect in the striatum could be a main contributor to its consolidation enhancing properties, although an indirect effect on the primary motor cortex through the M1-sensorimotor striatum-cerebellar circuit (Hikosaka et al., 2002) cannot be excluded. In this regard, caffeine-induced changes in synaptic efficiency within both the striatum (Di Filippo & Calabresi, 2016) and the primary motor cortex (Rioult-Pedotti et al., 1998, 2000) offer a plausible structural mechanism underlying the observed behavioural improvements. Given the ongoing uncertainty surrounding human motor learning mechanisms (Dayan & Cohen, 2011), caffeine represents an intriguing causal intervention tool since it is safe, readily administered, and, as demonstrated in the current experiment, capable of altering motor skill learning. Additionally, caffeine can directly modulate striatal activity, which is typically only indirectly accessible with non-invasive stimulation (Avissar et al., 2017). Therefore, future studies integrating caffeine administration with neuroimaging may represent a promising avenue for exploring the neural mechanisms underlying procedural memory consolidation.

Finally, the current experiment solely assessed one aspect of consolidation, namely offline learning as reflected by improvements in performance. However, consolidation is also characterized by the stabilization of memories, reflected in an increased robustness to interferences after learning a new task (Robertson, 2009) or a resistance to degradation over time (McCoy & Strecker, 2011). Importantly, the stabilization of procedural memories does not require sleep (Muellbacher et al., 2002; Walker et al., 2003) while offline learning does (Smith & Macneill, 1994; Stickgold et al., 2000; Stickgold & Walker, 2007), suggesting distinct

mechanisms underlying these phenomena. Since performance was only assessed with a single, short time interval between sessions and without a retroactive interference task, it remains unclear whether caffeine benefits both offline learning and memory stabilization. This distinction has significant practical implications, as enhanced offline learning may have limited value if performance improvements are temporary or easily disrupted. Without incorporating retroactive interference paradigms and longer intervals between assessments, it remains unclear whether caffeine-related performance gains reflect robust consolidation or transient, unstable improvements.

Conclusion

This study provides preliminary evidence that moderate caffeine intake following learning may support procedural memory consolidation, particularly by enhancing response speed and maintaining accuracy across days. While the expected three-way interaction was not observed, crucial two-way interactions suggest that 80mg of caffeine can improve implicit learning. However, the absence of similar effects at a higher dose, combined with diminished benefits among habitual caffeine users, indicates that these enhancements are both dose-sensitive and individual-dependent. Crucially, the magnitude of these effects was modest, and potential trade-offs, such as caffeine's disrupting impact on sleep, must be considered. Overall, while post-learning caffeine shows promise as a memory enhancer, its practical utility remains limited without further validation across populations, task types, and retention intervals.

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Appendix A

Group Comparisons on Covariates

Figure A1

Covariates per Group

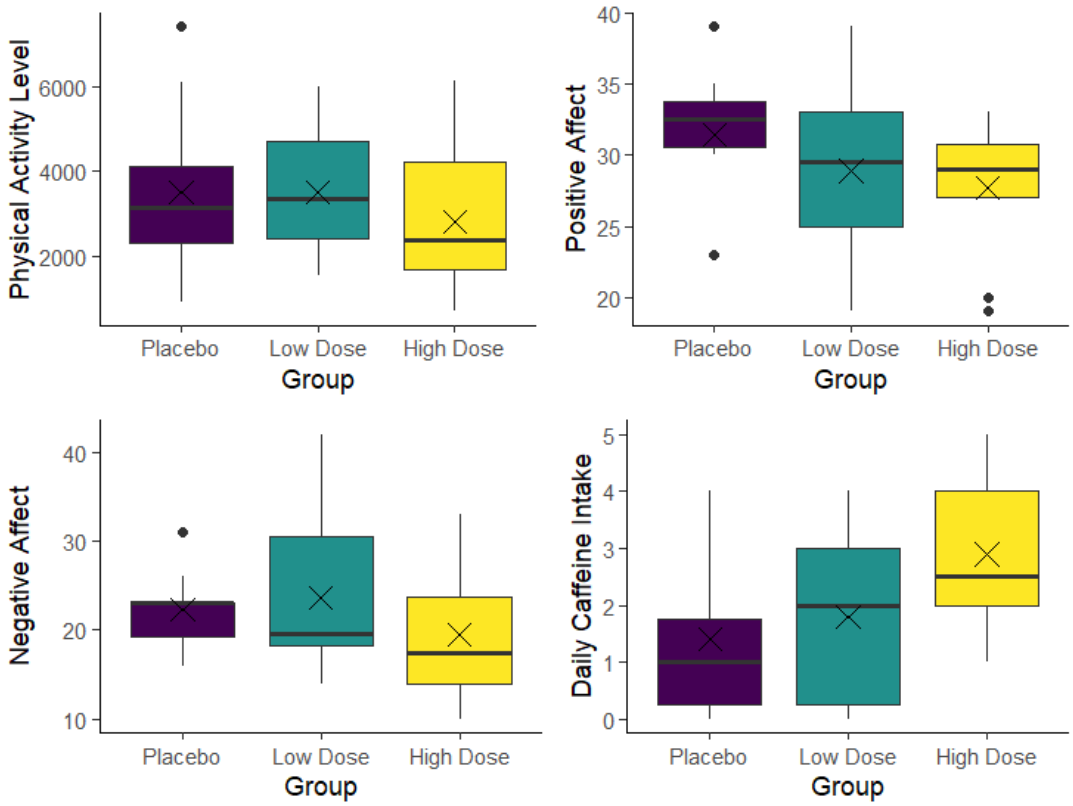


Table A1

Formal Comparisons Between Groups on Covariates

Covariate	Test Statistic	*p*-value
Physical Activity Level	$\chi^2(2, 30) = 0.78$	.68
Positive Affect	$\chi^2(2, 30) = 3.17$	.21
Negative Affect	$\chi^2(2, 30) = 1.82$	.40
Daily Caffeine Consumption	$\chi^2(2, 30) = 4.85$	.09

Note. A Kruskal-Wallis rank sum test was preferred because the assumptions of normality and homoscedasticity required for ANOVA were violated.

Appendix B

Psychometric Properties of the SRTT

Figure B1

Test-Retest Reliability (Overall and by Group)

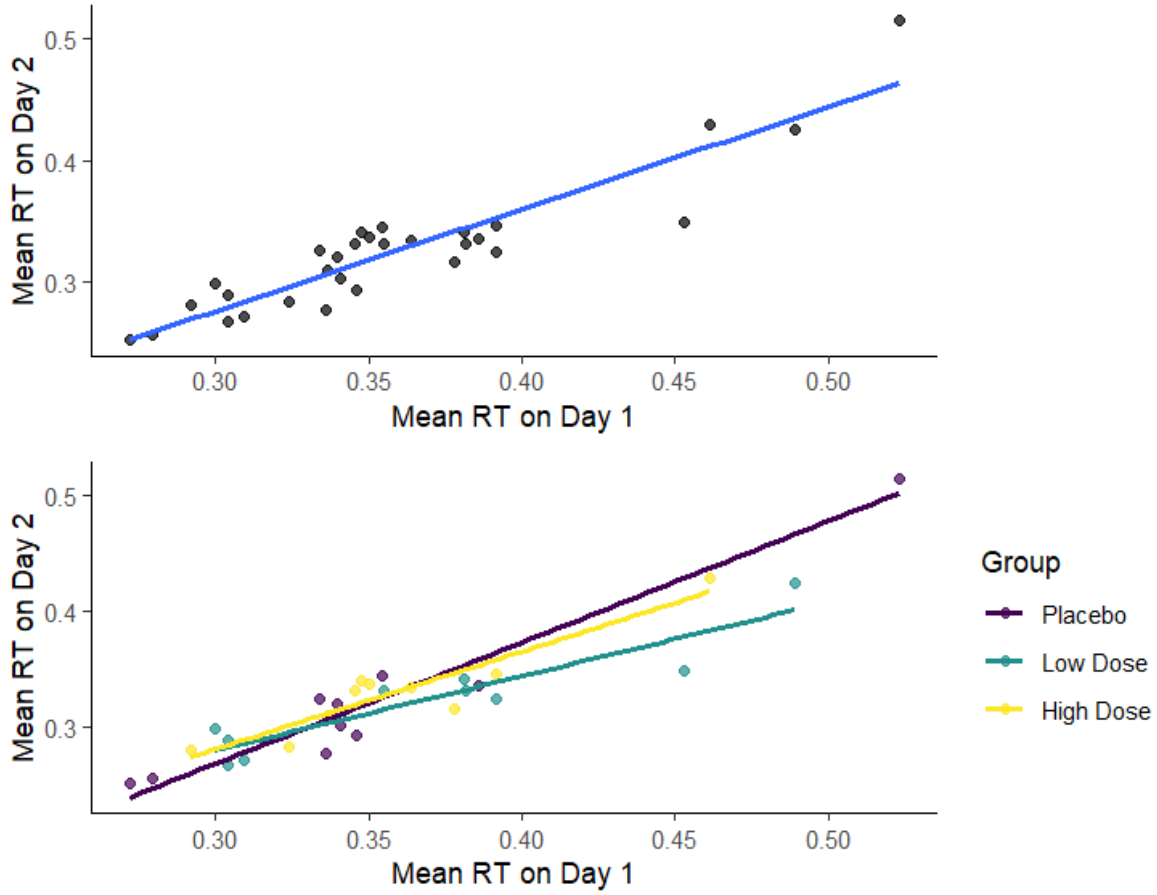


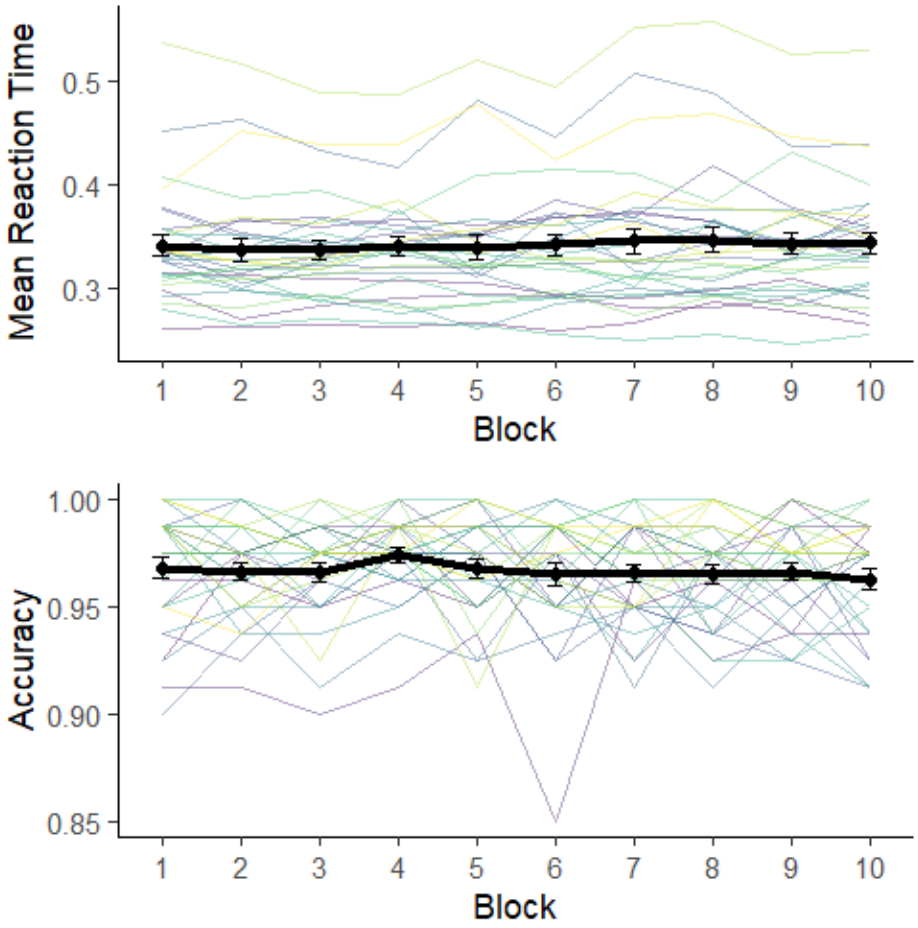
Table B1

Test-Retest and Split-Half Reliability

Metric	Value	95% CI
Test-Retest Reliability (All participants)	*r* = .92	[.84, .96]
Test-Retest Reliability (Placebo)	*r* = .97	[.87, .99]
Test-Retest Reliability (Low Dose)	*r* = .92	[.70, .98]
Test-Retest Reliability (High Dose)	*r* = .92	[.70, .98]
Split-Half Reliability	*r* = .62	[.60, .63]

Figure B2

Mean Reaction Time and Accuracy per Block



Note. Although a formal GLMM analysis indicated a statistically significant effect of block number on RT, β = 0.0004, SE = 0.002, $t(23996) = 2.272$, $p = .023$, the magnitude of this effect was negligible (an estimated increase of 0.4ms per block). Block number did not significantly predict trial accuracy, β = 0.016, $SE = 0.013$, $z = 1.26$, $p = .208$.

Appendix C

Hypothesis 1 Model Diagnostics and Selection

Figure C1

LMM Diagnostics

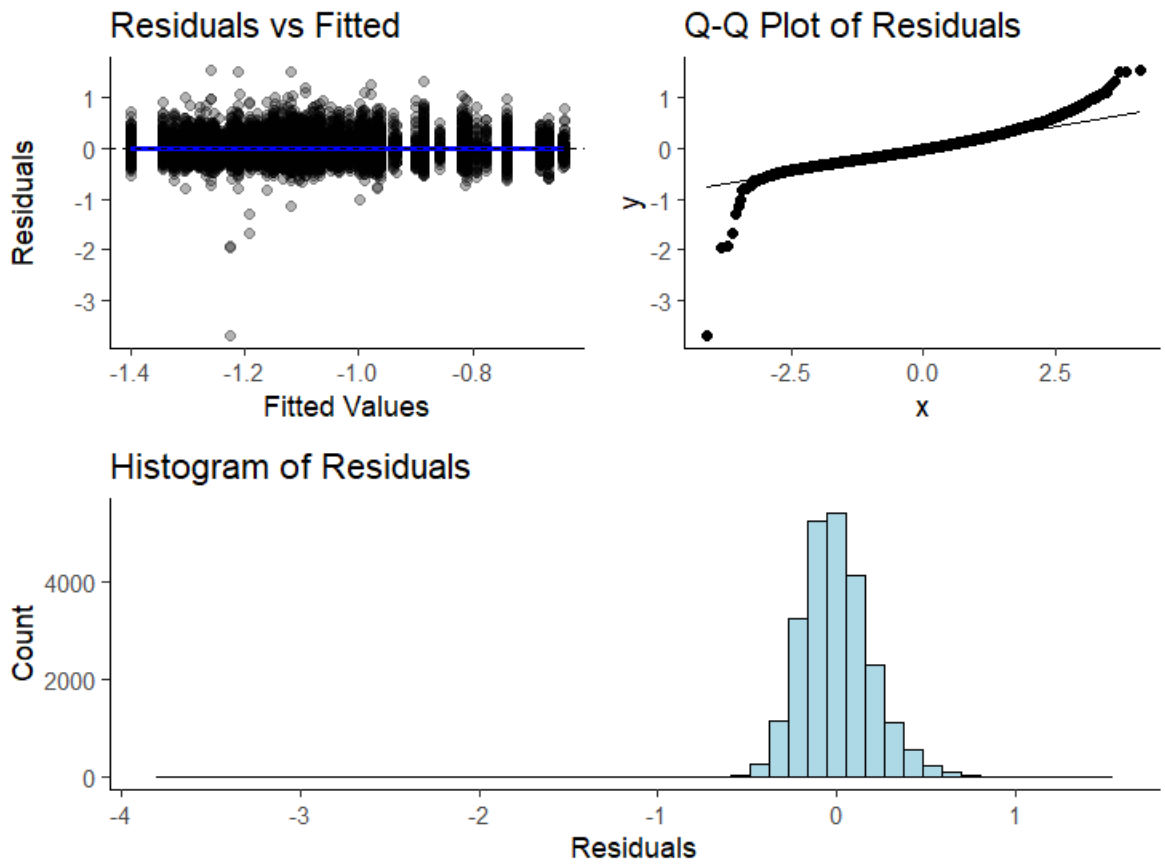


Figure C2

LMM Bootstrapped Regression Lines

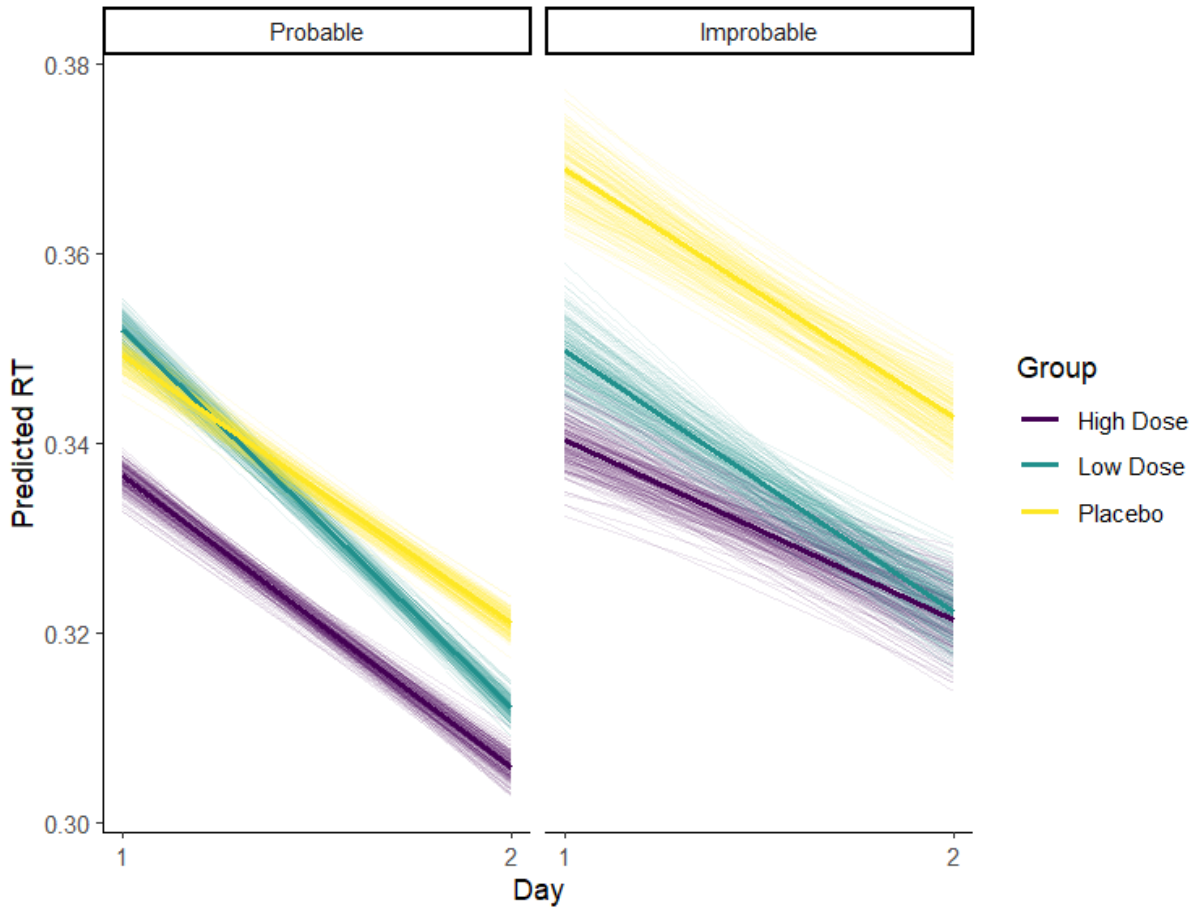


Figure C3

GLMM Diagnostics

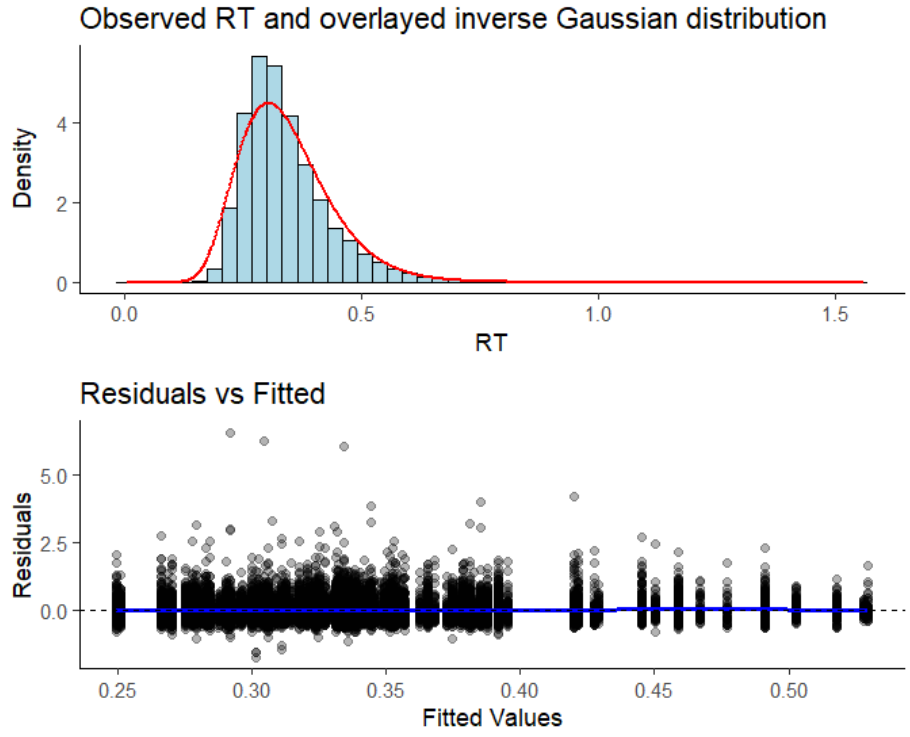


Table C1

GLMM VIF per Term

Term	VIF
Sequence	4.01
Day	3.52
Group	2.55
Regular Physical Activity	1.37
Baseline Positive Affect	1.31
Baseline Negative Affect	1.44
Sequence x Day	3.78
Day x Post-Test Sleep Duration	2.69
Day x Regular Caffeine Intake	1.94
Day x Group x Post-Test Positive Affect	2.73
Day x Group x Post-Test Negative Affect	1.66
Sequence x Group	5.52
Day x Group	7.04
Sequence x Day x Group	5.07

Table C2

GLMM Fit Evaluation

Model	Formula [a]	R^2	AIC	RMSE	Converged
Full Model	RT ~ Sequence * Day * Group + Regular Physical Activity + Post-Test Sleep Duration:Day + Regular Caffeine Intake:Day + Baseline Positive Affect + Post-Test Positive Affect:Day:Group + Baseline Negative Affect + Post-Test Negative Affect:Day:Group + (Day * Sequence | ID)	.006	-61389.23	0.077	Yes
Model 1.0	RT ~ Sequence * Day * Group + Regular Physical Activity + Post-Test Sleep:Day + Regular Caffeine	.005	-61395.25	0.077	Yes

	Intake:Day + Baseline Positive Affect + Post-Test Positive Affect:Day:Group + Baseline Negative Affect + Post-Test Negative Affect:Day:Group + (Day + Sequence | ID)				
Model 1.1	RT ~ Sequence * Day * Group + Regular Physical Activity + Post-Test Sleep:Day + Regular Caffeine Intake:Day + Baseline Positive Affect + Post-Test Positive Affect:Day:Group + Baseline Negative Affect + Post-Test Negative Affect:Day:Group + (Day | ID) + (1 | Sequence:ID)	.005	-61395.25	0.077	Yes
Model 1.2	RT ~ Sequence * Day * Group + Regular Physical Activity + Post-Test Sleep:Day + Regular Caffeine Intake:Day + Baseline Positive Affect + Post-Test Positive Affect:Day:Group + Baseline Negative Affect + Post-Test Negative Affect:Day:Group + (Day | ID)	NA	NA		No
Model 1.3	RT ~ Sequence * Day * Group + Regular Physical Activity + Post-Test Sleep:Day + Regular Caffeine Intake:Day + Baseline Positive Affect + Post-Test Positive Affect:Day:Group + Baseline Negative Affect + Post-Test Negative Affect:Day:Group + (1 | ID)	NA	NA		No
Model 1.4	RT ~ Sequence * Day * Group + Regular Physical Activity + Post-Test	NA	NA		No

	Sleep:Day + Regular Caffeine Intake:Day + Baseline Positive Affect + Baseline Negative Affect + Post-Test Negative Affect:Day:Group + (1| ID)			
Model 1.5	RT ~ Sequence * Day * Group + Regular Physical Activity + Regular Caffeine Intake:Day + Baseline Positive Affect + Baseline Negative Affect + Post-Test Negative Affect:Day:Group + (1| ID)	NA	NA	No
Model 1.6	RT ~ Sequence * Day * Group + Regular Caffeine Intake:Day + Baseline Positive Affect + Baseline Negative Affect + Post-Test Negative:Day:Group + (1| ID)	NA	NA	No
Model 1.7	RT ~ Sequence * Day * Group + Regular Caffeine Intake:Day + Baseline Positive Affect + Baseline Negative Affect + (1| ID)	NA	NA	No
Model 1.8	RT ~ Sequence * Day * Group + Baseline Positive Affect + Baseline Negative Affect + (1| ID)	NA	NA	No
Model 1.9	RT ~ Sequence * Day * Group + Baseline Positive Affect + (1| ID)	NA	NA	No
Model 1.10	RT ~ Sequence * Day * Group + (1| ID)	NA	NA	No

Note. The model retained for hypothesis testing is marked in bold.

[a] Models specified using lme4 syntax. The equation for the retained model can be seen in Equation (C1).

$$
\begin{aligned}
Y_{ij} = \beta_0 + \beta_1 \cdot \text{Sequence}_i + \beta_2 \cdot \text{Day}_i + \beta_3 \cdot \text{Group}_j \\
+ \beta_4 \cdot \text{Sequence}_i \cdot \text{Day}_i + \beta_5 \cdot \text{Sequence}_i \cdot \text{Group}_j + \beta_6 \cdot \text{Day}_i \cdot \text{Group}_j \\
+ \beta_7 \cdot \text{Sequence}_i \cdot \text{Day}_i \cdot \text{Group}_j \\
+ \beta_8 \cdot \textit{R}\text{egular Physical Activity}_j + \beta_9 \cdot \text{Baseline Positive Affect} + \beta_{10} \\
\cdot \text{Baseline Negative Affect}_j \\
+ \beta_{11} \cdot \text{Day}_i \cdot \text{Post} - \text{Test Sleep Duration}_i + \beta_{12} \cdot \text{Day}_i \\
\cdot \text{Regular Caffeine Intake}_i \\
+ \beta_{13} \cdot \text{Day}_i \cdot \text{Post} - \text{Test Positive Affect}_i \cdot \text{Group}_j + \beta_{14} \cdot \text{Day}_i \cdot \text{Post} \\
- \text{Test Negative Affect}_i \cdot \text{Group}_j \\
u_{0j} + u_{1j} \cdot \text{Day}_i + u_{2j} \cdot \text{Sequence}_i + u_{3j} \cdot (\text{Day}_i \cdot \text{Sequence}_i) + \varepsilon_{ij}
\end{aligned}
\tag{C1}
$$

Where:

Y_{ij}: RT for trial i of participant j

Sequence_i: Task sequence (A / B)

Day_i: Session, dummy coded (Day 1 = 0, Day 2 = 1)

Group_j: Treatment group (Placebo / Low Dose / High Dose)

Regular Physical Activity, Baseline Positive Affect, Baseline Negative Affect: baseline covariates (*z*-standardized)

Post-Test Positive Affect, Post-Test Negative Affect, Post-Test Sleep Duration, Regular Caffeine Intake: post-test covariates (*z*-standardized)

u_{0j}, u_{1j}: Random intercept per participant

u_{1j}: Random slope for Day per participant

u_{2k}: Random slope for Sequence per participant

u_{3j}: Random slope for Day × Sequence per participant

ε_{ij}: Residual error