Acute cocoa flavanol intake does not modulate interference or inhibitory control: a randomised, controlled trial.

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Abstract

A growing number of research studies have examined the potential benefits of cocoa flavanols supplementation on neuro-physiological processes and cognitive enhancement. However, the results have led to limited evidence on both chronic and acute effects. This study investigated whether acute cocoa flavanol consumption might modulate interference and inhibitory control responses. In order to test the effects on cognition, we implemented the Flanker, Go/No-Go and Simon tasks. Thirty-six healthy university students, aged 19-29 years, completed this randomised, gender-balanced, double-blind, placebo-controlled, counterbalanced, crossover study. In three separate days, participants received drinks containing 622.5 mg (medium condition), 415 mg (low condition), and 0 mg (placebo) cocoa flavanols. They completed the experimental tasks one-hour post-ingestion. Compared to the placebo, neither the medium nor the low conditions significantly reduced reaction times and improved accuracy in the three tasks. We conclude that acute cocoa flavanol intake did not modulate interference or inhibitory control functions.

Keywords: cocoa flavanols, cognition, interference control, inhibitory control.

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Introduction

Known for their antioxidant properties, chocolate and cocoa-derived products are made of polyphenol compounds. Polyphenols comprise at least 8000 types of flavonoids and can be subdivided into flavones and flavanols (Bravo, 1998). A considerable percentage of flavonoids can be found in common foods, for instance, apples, grapes, red wine, blackberries, green and black teas, caffeine, legumes, and dark chocolate (Shen et al., 2022; Gu et al., 2004; Manach et al., 2004; Lazarus et al., 1999).

In recent years, experimental studies have examined the acute and chronic cocoa flavanol (CF) intake and their neuroprotective effects on promoting healthy ageing in non-clinical populations (Spencer, 2009) and on the progression of neuro-pathologies like Parkinson's and Alzheimer's diseases. For instance, Mastroiacovo et al. (2015) demonstrated that a daily CF intake generated in healthy elderly participants' improvements in processing speed, working memory mechanisms and executive functions. Desideri et al. (2012) reached similar conclusions in participants affected by mild cognitive impairment, besides a decrease in blood pressure and improvement in insulin sensitivity. In contrast, Camfield et al. (2012) and Crews et al. (2008) found no cognitive or behavioral effects.

CF is believed to exert neurobiological effects through direct and indirect mechanisms in the Central Nervous System and cardiovascular circulation (Sokolov et al., 2013). CF components are in fact capable of crossing the blood-brain barrier and reaching different cerebral areas, such as the hippocampus, cerebellum, striatum and cerebral cortex (Socci et al., 2017). According to Valente et al. (2009) and Nehlig et al. (2013), CF compounds induce

the production and expression of brain-derived neurotrophic proteins, which are fundamental to ensure neurogenesis, synaptic growth, neural survival and differentiation of new neurons.

Moreover, CF compounds are responsible for inducing indirect neurocognitive effects. Indeed, CF promotes vasodilation in the central and peripheral systems and reduces neuro-inflammation. In other words, better vasodilatation and neurotransmission encourage the production of the neurotransmitter endothelial nitric-oxide synthase (Martín et al., 2020). Following the augmented vasodilatation, it is possible to increase cerebrovascular perfusion, regulate blood pressure (Taubert et al., 2007) and improve vascular tone (Grassi et al., 2005).

Considering these neurobiological effects, whether CF consumption is associated with modulation of cognitive processes has been analysed. In a counterbalanced, double-blind, crossover study, Francis et al. (2006) investigated whether chronic CF consumption could determine an improvement in RT and an increment in the blood oxygenation level-dependent (BOLD). They implemented a high CF condition (516 mg CF) and low CF condition (39 mg CF) and instructed the participants to perform either a switch or non-switching task, while they underwent two fMRI sessions. The findings demonstrated a significant increment in the BOLD signal in the high CF dose compared to the low CF dose, with a peak two hours after ingestion. Despite these results, Francis et al. did not find any significant evidence that the increase in the BOLD determined cognitive improvements in the switching task.

A following study conducted by Lamport et al. (2015) replicated the findings from Francis et al. and restricted them to more specific cerebral areas, such as the anterior cingulate cortex and the central opercular cortex. However, it was not possible to determine any cognitive effects associated with the increased regional cerebral perfusion since the change in perfusion was assessed while participants were in a resting state.

In line with the previous findings, Decroix et al. (2016) found no significant benefits on cognitive functions, despite the positive physiological results. In their double-blind,

randomised, crossover experiment, they implemented the functional near- infrared light attenuation (NIRS) and used a high CF condition (900 mg CF) and placebo. Indeed, they showed that the 900 mg CF intake determined a significant increase in cerebral oxygenation level, although no significant impact was registered on the Stroop task.

Conversely, the randomised, double-blinded, crossover pilot study of Decroix et al. (2019) showed significant facilitation in executive functions alongside neurobiological indexes in type 1 diabetes patients and their matched control participants. They gave the participants a Flanker task two hours' post-ingestion and measured their BOLD response with fMRI. The results demonstrated a behavioural effect of the CF intake in the 900 mg CF drink condition compared to the placebo (15 mg CF). That is, the increased BOLD response in the supra-marginal gyrus of the parietal lobe and inferior frontal gyrus in both experimental groups determined faster reaction time (RT) on the Flanker task.

Another demonstration was provided by Field et al. (2011). They investigated the effects of CF consumption on cognitive performances as well as measures of visual processing, using dark chocolate (733 mg CF) as a high CF condition and white chocolate as placebo. It was hypothesised that the acute CF intake would determine an improvement in the test battery and differences overall between treatment conditions were found. Small significant effects were registered with the completion of the spatial working memory and the choice reaction time tasks. Greater improvements were registered in motion integration time threshold and visual contrast sensitivity tasks, which the hypothesis of a change in the retina blood flow post-CF intake could support. However, the placebo effect might represent a limitation of the study. That is, in this single-blinded study, the white chocolate was easily identifiable by the participants and, therefore, might have influenced their test performances.

Subsequently, two double-blind, counterbalanced, crossover experiments conducted by Altinok et al. (2022) tried to replicate the findings from Field et al. focusing only on visual

working memory and involving participants within the same age range as Field et al. In the first experiment, they measured recall precision while participants were passively maintaining the grating orientations in the working memory; in the second experiment, participants were required to update the content task actively through mental rotation of the information. Nevertheless, the results indicated that CF consumption did not significantly facilitate, in terms of RTs and accuracy, visual working memory recall performance or recall accuracy.

Scholey et al. (2010) found positive cognitive effects of CF intake s using the standardised cognitive demand battery (CDB) test of six cycles. Healthy young adults were involved in this double-blinded, crossover study. Participants completed the Serial Threes and Sevens Subtraction tasks, where they had to count backwards in trees and then sevens from a random starting number between 800 and 900; then, they were presented with a rapid visual information processing (RVIP) task with a series of digit strings for targets; and, finally, participants completed a self-rated mental fatigue visual scale. The results demonstrated mixed effects. On the one hand, they found no significant improvements in the Serial Sevens task or in the accuracy rate of the RVIP task. On the other hand, CF consumption facilitated the Serial Threes task in both low (520 mg CF) and high CF conditions (994 mg CF) compared to the control drink. Moreover, participants in the high dose CF condition became significantly faster on the RVIP task in the third and fourth cycles of CBT than in the low and control conditions. The self-rated mental fatigue was significantly reduced only in the CF low dose. Massee et al. (2015) partially confirmed the acute CF findings from Scholey et al. showing an improvement in the first cycle of the Serial Seven Subtraction task in the 250 mg CF condition compared to the placebo.

In another randomised, double-blind, parallel group study, Pase et al. (2013) did not find any CF supplementation effect on cognitive performance, in line with Crews et al.

(2008). However, a significant effect was found on the sub-chronic CF effect on the self-reported mood scale scores: participants in the high CF condition (500 mg CF) reported feeling more calm and content than participants in the low CF condition (250 mg CF) and the control group after 30 days of CF consumption. Still, the presence of a standardised lunch-break between the CF intake and the cognitive assessment represents a critical point since the postprandial glucose state might have attenuated the effect of CF compounds in the participants' organisms.

Finally, Karabay et al. (2018) speculated that CF acute consumption might facilitate spatial and temporal attention. Instead of the CDB, in the randomised, double-blind, counterbalanced, crossover design, the researchers elaborated a visual search task and a rapid visual presentation (RSVP) task, which integrated attentional blink and temporal integration mechanisms. In line with previous studies (Scholey et al., 2010), the performance on the RSVP task was not enhanced by CF supplementation in the high CF dose group (747 mg CF) nor low CF dose group (374 mg CF) compared to the placebo and baseline conditions. Meanwhile, the visual search task revealed different results and improved efficiency. On the one hand, CF did not improve the accuracy of the visual search task since a ceiling effect occurred in all conditions. On the other hand, RT was significantly affected by CF supplementation, and participants reported faster RT in the completion of the visual search task without losing accuracy.

Taken together, mixed evidence was found in the literature review. The results indicated that acute CF consumption can either modulate or left unchanged cognitive functions. As stated by Barrera-Reyes et al. (2020), the inconsistency in the empirical findings might be due to variation in sample sizes and statistical power across studies, as well as CF dose content, inter-individual factors involved in the absorption and response to CF intake, and the implementation of different study designs.

In the current study, we aim to assess whether CF influences interference and inhibitory control responses in a healthy and young sample size using Flanker, Go/No-Go and Simon tasks. While Decroix et al. (2019) reported significant results as measured by the Flanker tasks, no previous research has looked at the acute CF effects on Go/No-Go and Simon tasks.

Modified versions of the Flanker (Eriksen & Schultz, 1979) and Simon tasks (Simon et al., 1970) were implemented in the study to examine interference control. The interference effect is given when a task-irrelevant stimulus is presented, activating an automatic response. Therefore, a cognitive control response is applied to suppress the automatic response and provide the correct task-relevant response (Diamond, 2013). In the Flanker task, the target and flankers are presented in a single line across congruent and incongruent trials. The interference control typically occurs in incongruent trials when flankers point in the opposite direction of the target and affect participants' responses. In the Simon task, faster and more accurate responses are registered in congruent trials when the side of the arrow presentation corresponds to the side of the keypress on which a response is expected (e.g., a leftward arrow pointing to the left). Alternatively, the interference effect is evident across incongruent trials when arrows location mismatches the side of the correct keypress (e.g., a rightward arrow pointing to the left).

Moreover, a modified version of the Go/No-Go task (Donders, 1969) was used to detect inhibitory control and measure participants' ability to withhold their responses. In order to ensure a high degree of conflict in participants' responses, we implemented more Go-trials than No-Go trials. The outcomes measured were RT of the responses to Go-stimuli and accuracy, defined by commission errors as the number of No-Go stimuli which were falsely responded to.

We firstly hypothesise that acute CF intake would facilitate performances on the Flanker task. The prediction is that RT becomes faster and accuracy rate increases in both congruent and incongruent trials. For our second hypothesis, we address whether the Go/No-Go task is modulated by acute CF consumption by predicting that RT on Go-trials becomes faster and commission errors on No-Go trials decrease. The third hypothesis investigates whether acute CF intake enhances interference control in the Simon task. We predict that RT becomes faster and accuracy rate increases in congruent and incongruent trials.

Method

Participants

Thirty-six university students (18 females; 18 males) participated in the study (*M* = 21.5 years, *SD* = 2.6, range = 19-29). A priori analysis conducted using G*Power software determined that an effect size with *d* = 0.25 would require a sample size of 36 to obtain actual power = 0.9 when α = 0.05 and critical *F* = 3.13. Recruitment took place through the SONA system, and participants received compensation for their engagement in this study, either in course credits or money (€ 45 given after the completion of the third session). The study obtained approval from the University of Groningen's ethics committee of the Psychology Department, Faculty of Behavioural and Social Sciences and was conducted in accordance with the Declaration of Helsinki (2008). As part of the written informed consent given before the start of the experiment, participants were not previously diagnosed with any neurological or psychiatric disorders or any health disorder affecting metabolism and vascular diseases. Moreover, they did not adhere to a medically restricted diet nor take prescription medication or vitamin supplements, herbal extracts or illicit drugs. They were not pregnant or breastfeeding and did not have wheat, gluten, soy, milk, egg, fish, shellfish or tree nuts allergies.

Experimental product

The cocoa and alkalized powders were provided free of charge by the Barry Callebaut company. This company was not sponsored or involved in the current study. The three experimental drinks were placebo, low CF condition and medium CF condition. The placebo consisted of 9 g alkalized cocoa powder; the low CF condition included 4 g alkalized cocoa powder and 5 g high-flavanol powder; the medium CF condition contained 1.5 g alkalized cocoa powder and 7.5 g high-flavanol powder. The alkalized cocoa powder contained no

flavanols and was used to conceal colour differences. The several powders were served with 200 ml tap water. The nutritional composition details of the drinks are illustrated in Table 1.

Table 1

Nutritional Composition of the Experimental Drinks

	Medium CF condition			Low CF condition			Placebo
	7.5 g high-flavanol cocoa powder	1.5 g alkalized cocoa powder	CF-total	5 g high-flavanol cocoa powder	4 g alkalized cocoa powder	CF-total	9 g alkalized cocoa powder
Flavanols (mg)	622.5	0	622.5	415	0	415	0
Energy (kcal)	25.8	4.6	30.36	17.2	12.6	29.8	27.4
Protein (mg)	1680	333	2013	1120	888	2008	1998
Fat (mg)	1050	165	1215	700	440	1140	990
Caffeine (mg)	15	3	18	10	8	18	18
Theobromine (mg)	157.5	31.2	188.7	105	84	189	187.2
Water (ml)	200			200			200

Materials and apparatus

The experimental tasks were programmed in OpenSesame 3.1.9 and executed under the Windows 7 operating system. The tasks were presented on a 22" CRT monitor (Iiyama Prolite G2773HS) with a refresh rate of 100Hz. The screen resolution was set to 1024x768 pixels for each task.

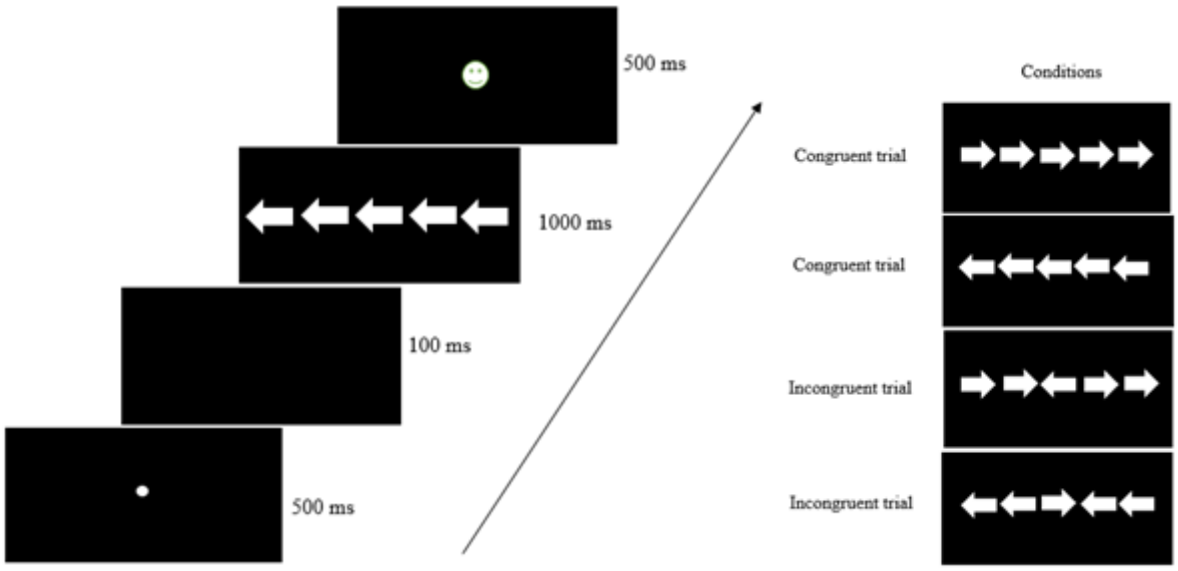
In the questionnaire given before the start of the experiment, participants were asked to indicate the session type, their gender, their weight in kg and their height in cm. Next, it was asked: "In the last three months, how often did you drink red wine?"; "In the last three months, how often did you drink green tea?"; and "In the last three months, how often did you consume dark chocolate?". For each of these questions, it was possible to indicate the number of times the product consumed and check one of the three boxes: "in a day", "in a week", and "in a month". The following open questions were then asked: "Do you smoke cigarettes ?"; "Did you smoke cigarettes in the last twelve hours?"; "Did you drink alcohol yesterday?"; "Did you drink more than two glasses of alcoholic drinks yesterday evening?"; "Did you drink coffee this morning?"; "Do you have any vascular disease?"; "Do you have any disease affecting your metabolism?"; "Do you have any neurological or psychiatric disease?"; "Do you follow a medically restricted diet?"; "Are you pregnant?"; "Do you use contraceptive pills?"; "Do you take vitamin supplements?". The following answers were given to choose from: "Yes"; "No".

In the Flanker task, stimuli implemented for targets and flankers were arrows oriented horizontally and presented in white on a black background (see Figure 1). They were pointing in the left and right direction. In each display, a line of five arrows was presented in the centre of the screen. The width of each arrow was 39 pixels (1.61˚ of visual angle) and the length was 60 pixels (3.11˚ of visual angle). The target was the third arrow shown with two flankers on each side. The task included congruent and incongruent trials. In the congruent

trials, the target and flankers were pointing in the same direction, “<<<<<” or “>>>>>”. In the incongruent trials, the target was pointing to the left and the flankers in the right direction (i.e. >><>>), or the target was pointing to the right direction, while the flankers to the left (i.e. <<><<).

Figure 1

The Flanker Task



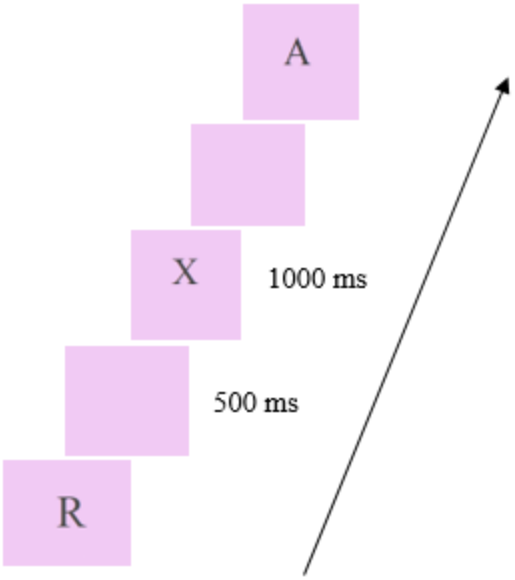
Note. An example of the Flanker trial procedure is represented. Flankers and targets are pointing in the left direction. The stimuli display is followed by positive feedback. Task conditions are reported on the right side.

The Go/No-Go task included 11 letters as visual stimuli and used them as targets and distractors. The pixels for each stimulus were 18*18 (.93˚ * .74˚ of visual angle). They were presented in black in the centre of the screen on a decade background (see Figure 2). The task was constituted of Go and No-Go trials. The letters “W”, “R”, “Y”, “I”, “O”, “A”, “D”, “G”,

"J", and "K" were used as targets in the Go trials. The letter "X" was the distractor implemented in the No-Go trials.

Figure 2

The Go/No-Go task



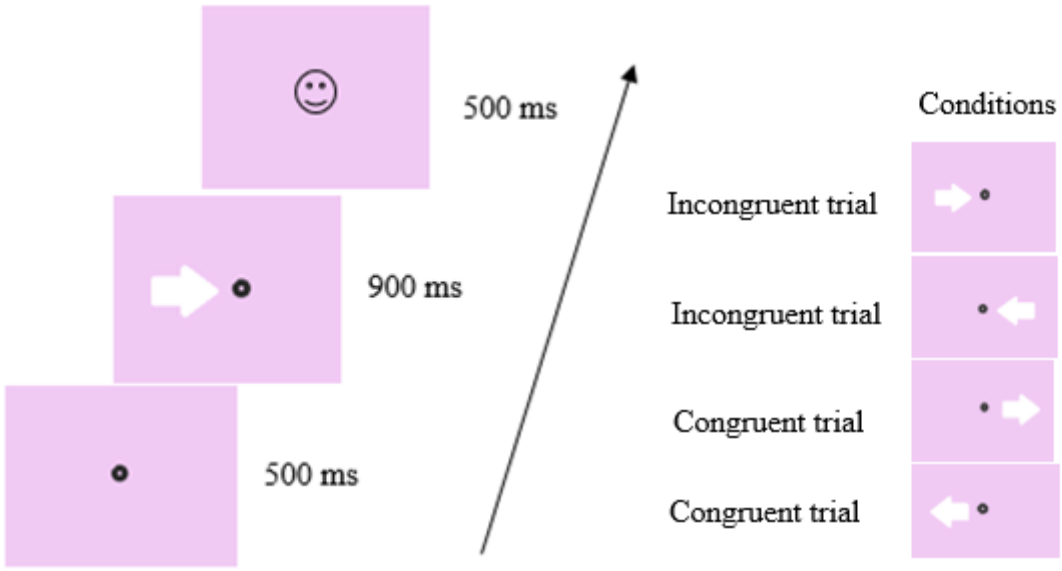
Note. An illustration of the possible targets and distractors is presented on the screen in the Go/No-Go task.

Regarding the Simon task, two target stimuli were shown simultaneously on a decade background: a black fixation dot and a white arrow. The width of the arrow was 26 pixels (1.07˚ of visual angle) and the length was 44 pixels (2.28˚ of visual angle). The fixation dot was displayed in the centre of the screen, and the arrow was oriented horizontally and positioned either on the left or right side of the dot. The arrow was identified as the target stimulus and displayed as pointing to the dot or in the opposite direction. The task consisted of congruent and incongruent trials. For congruent trials, the arrows were leftward to the dot and it pointed in the left direction; alternatively, the arrows were rightward and it pointed to

the right. For incongruent trials, leftward arrows pointed in the right direction and rightward arrows pointed to the left (see Figure 3).

Figure 3

The Simon Task



Note. On the left side of the panel there is an example of incongruent trial of the Simon task. The presentation of the stimuli is followed by positive feedback. On the right side, the four possible conditions of congruent and incongruent trials possible are shown.

Procedure

To prevent diurnal effects, participants attended the lab on three different days and at fixed times: at 9:00, 10:00, 11:00, 12:00, 13:00 or 14:00. The sessions were separated by a wash-out period of three to seven days. Twelve hours prior to the experiment, participants were invited to abstain from smoking, take vitamin supplements and avoid consuming flavonoid products such as caffeine, alcohol, green and black tea. On the session day, a first researcher provided the experimental drinks to the participants, in addition to the informed consent, the information about the study and instructions of the cognitive tasks. Participants

were not aware of the type of drinks they received. After the CF or placebo drink intake, the researcher invited the participants to wait in the university building for 60 min and abstain from any food or drink except water. This choice was made to promote a maximal assimilation of CF by the body and to avoid a postprandial effect in the participants. A second researcher, not involved in the drink assignment, administered the experimental tasks in the lab. Participants were seated in a lighted room approximately 60 cm away from the computer screen. Before the beginning of the experiment, a questionnaire was given to the participants to determine the quantity of flavonoid products they might have consumed in the recent past. The questionnaire took approximately five min and participants completed it in each session.

The experiment consisted of three tasks given to the participants in the same order for every session: Flanker task, Go/No-Go task, and Simon task. Instructions were repeated before the start of each task and participants were invited to respond as quickly and accurately as possible. For each task, a series of practice trials were completed before starting the actual experimental blocks and were eventually excluded from the statistical analysis. Participants were not allowed to repeat the practice phases.

The experiment sequence started with the Flanker task. A trial began with the onset of the fixation dot at the centre of the screen for 500 ms; next, a blank layout was shown for 100 ms, followed by the stimuli display. Participants were required to report the direction of the target for each line of arrows while ignoring the flankers. When the target pointed to the left, they had to press “Z” on their keyboard; when it pointed to the right, they had to press “M”. The stimuli display appeared on the screen for 1000 ms, after which a positive or negative feedback screen was presented for 500 ms and followed again by a blank screen for 500 ms. The task was divided into four identical blocks and each of them contained 100 trials for a total of 400 trials. Eight practice trials preceded them. Trials were presented in randomized

order within each block. In 50% of the trials, the target and flankers pointed in the same direction; in the other 50%, the target indicated a different direction than the flankers.

After the completion of the first task, participants started the Go/No-Go task. Each trial started with a blank screen with a duration of 500 ms, followed by a letter presented in the middle of the screen. Participants had to press “M” on their keyboard whenever a target was presented. When the distractor was shown on the screen, they were instructed to withdraw their responses. After 1000 ms, the letter disappeared and the screen returned blank. There were seven practice trials followed by one experimental block made of 400 trials arranged in random order. The block consisted of 270 targets, 67.5% Go trials, and 130 distractors, 32.5% No-Go trials.

Following, the Simon task was administered to the participants. The trial began with a fixation dot presented at the centre of the screen for 500 ms, followed by an arrow and fixation dot. Within a time-window of 900 ms, participants had to press “A” on their keyboard whenever the arrow pointed to the left and “L” when pointed to the right. Afterwards, a positive or negative feedback appeared on the screen for 500 ms. Participants were allowed to take a short break between blocks. The task started with eight practice trials. Four experimental blocks came next with 100 trials in each block for a total of 400 randomized trials. Within a block, there was an equal number of congruent and incongruent trials. The total duration of three experimental tasks was approximately 45 min. Each participant was thanked for their time and effort at the end of the experiment.

Design and statistical analysis

This study made use of a randomized, gender-balanced, double-blind, placebo-controlled, counterbalanced, crossover, repeated measures design. Each participant received the three experimental drinks and completed the three cognitive tasks each time.

A 3 (condition: medium CF, low CF, and placebo) * 2 (trial type: congruent, incongruent) repeated measures of analysis of variance (RM-ANOVA) design was implemented in the Flanker task to investigate CF effect on RT. Next, we performed the same RM-ANOVA to assess CF modulation in accuracy.

We conducted a one-way RM-ANOVA (condition: medium CF, low CF, and placebo) on the Go trials to assess CF effect on RT. Following, a one-way RM-ANOVA (condition: medium CF, low CF, and placebo) was performed in the No-Go trials to determine commission errors after CF supplementation.

A 3 (condition: medium CF, low CF, and placebo) * 2 (trial type: congruent, incongruent) RM-ANOVA design was used to analyse performance in the Simon task in terms of RT. The same RM-ANOVA design was then implemented to measure accuracy after CF intake.

All analyses were performed using JASP software (Version 0.17.2; JASP Team, 2023).

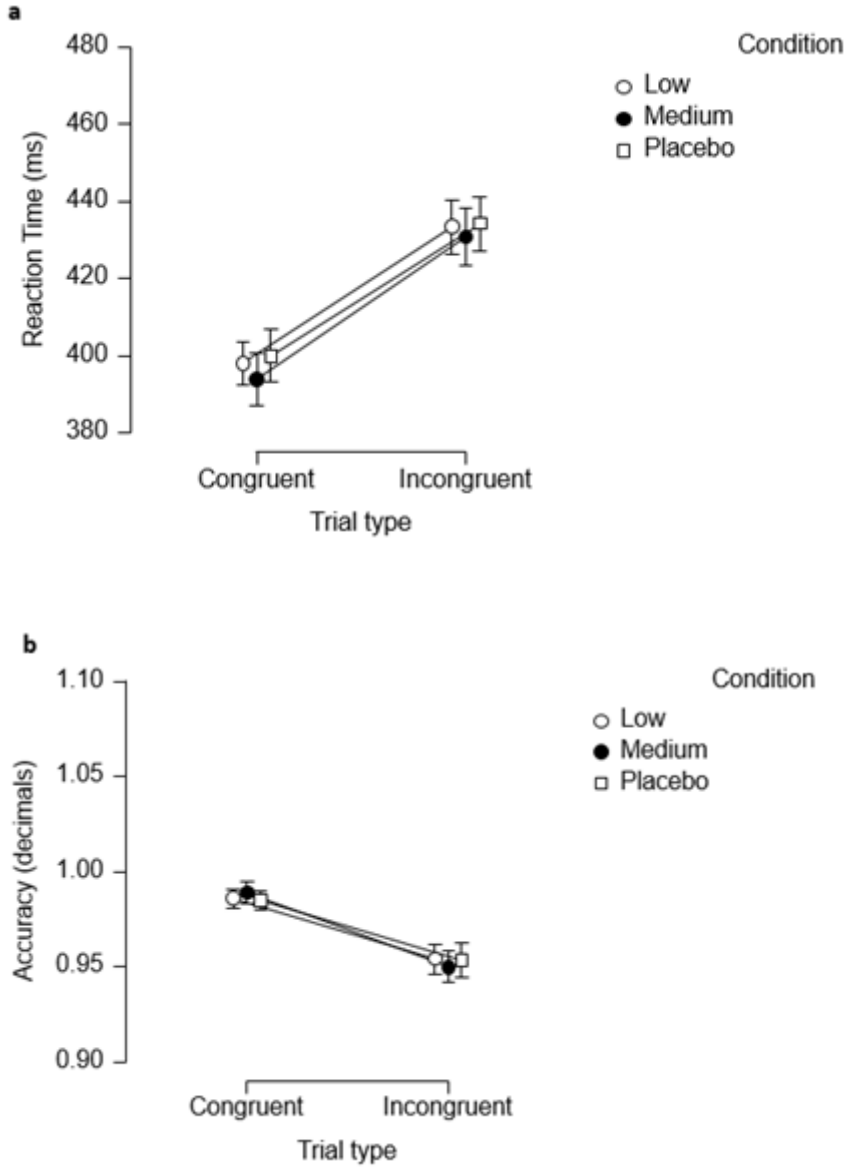
Results

We aggregated the data and established that trials with RT higher than 100ms were considered extremely slow and therefore not exploitable for the analysis. Missing responses were not excluded. Before conducting RM-ANOVAs, we checked whether the variables were normally distributed using Q-Q plots and normality was met. Sphericity was assessed for the variables involved in RM-ANOVAs and there was no evidence that the assumption was violated. Tukey (HSD) test was used for pairwise comparisons in post hoc analysis. Statistically significance was determined at $p < .05$. Descriptive statistics are shown in Table 2 for the Flanker task, in Table 3 for the Go/No-Go task and in Table 4 for the Simon task.

For the first hypothesis, a two-way RM-ANOVA (see **Appendix A**) indicated that the main effect of CF condition on RTs of the Flanker task was non-significant, $F(2,70) = 0.48$, $p = .618$, $\eta^2_p = .01$, but a significant main effect of trial type was found, $F(1,35) = 381.18$, $p < .001$, $\eta^2_p = .92$. Further post-hoc comparisons revealed that participants' responded faster in congruent than incongruent trials, $M = -35.56$, $SD = 1.82$. The interaction effect between CF condition and trial type was non-significant, $F(2,70) = 0.52$, $p = .599$, $\eta^2_p = .02$. That is, the RT did not become faster in congruent or incongruent trials following the CF intake. Mean accuracy across all conditions was 98.7% in congruent trials and 95.3% in incongruent trials. A second two-way RM-ANOVA on accuracy rate revealed a non-significant main effect of CF conditions, $F(2,70) = 0.04$, $p = .963$, $\eta^2_p = .00$, but a significant main effect of trial type was found, $F(1,35) = 74.46$, $p < .001$, $\eta^2_p = .68$. Indeed, accuracy was higher in congruent than incongruent trials, $M = 0.03$, $SD = 0.00$. Finally, the interaction of CF condition and trial type did not modulate accuracy significantly, $F(2,70) = 1.35$, $p < .267$, $\eta^2_p = .04$ (see Figure 4).

Figure 4

Cognitive Results in the Flanker Task



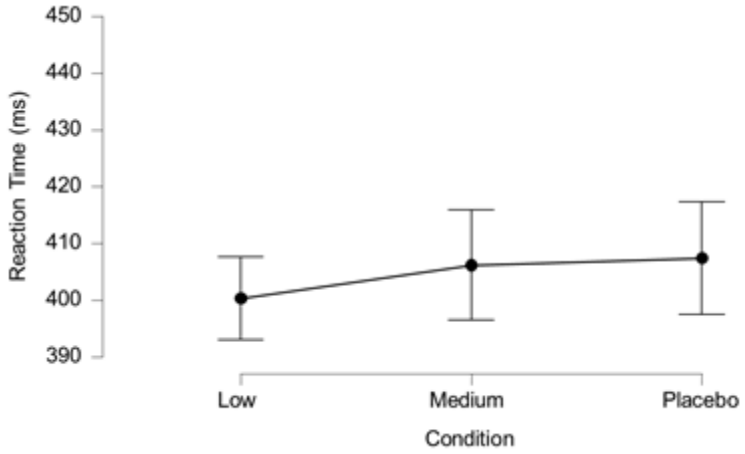
Note. Mean change in RT (y-axis, in ms) among the three CF conditions in congruent and incongruent trials is illustrated (a). The change in accuracy rate (y-axis, in decimals) across trial types following the consumption of CF drinks is reported (b). The error bars represent 95% confidence intervals around the mean.

Regarding our second hypothesis, one-way RM-ANOVA results (see **Appendix B**) revealed a non-significant effect of CF condition on RT in the Go trials, $F(2,70) = 0.72$, $p = .492$, $\eta^2_p = .02$. Moreover, a second one-way RM-ANOVA on the commission errors for the No-Go trials suggested a non-significant effect of CF condition, $F(2, 70) = 0.\ 98$, $p = .381$, $\eta^2_p = .03$ (Figure 5).

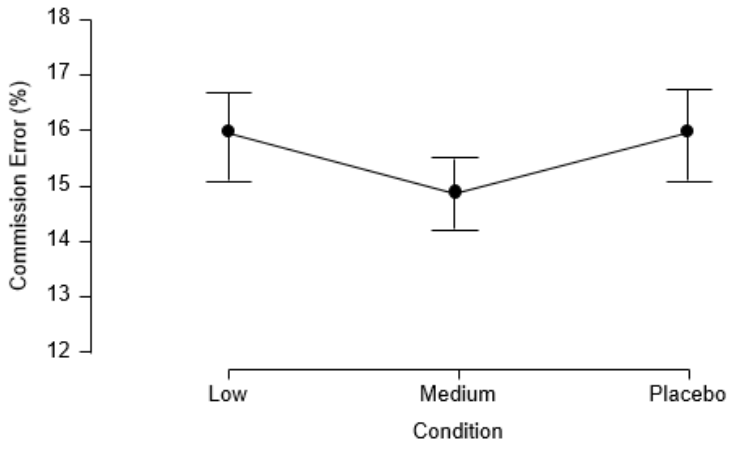
Figure 5

Cognitive Results in the Go/No-Go Task

a



b

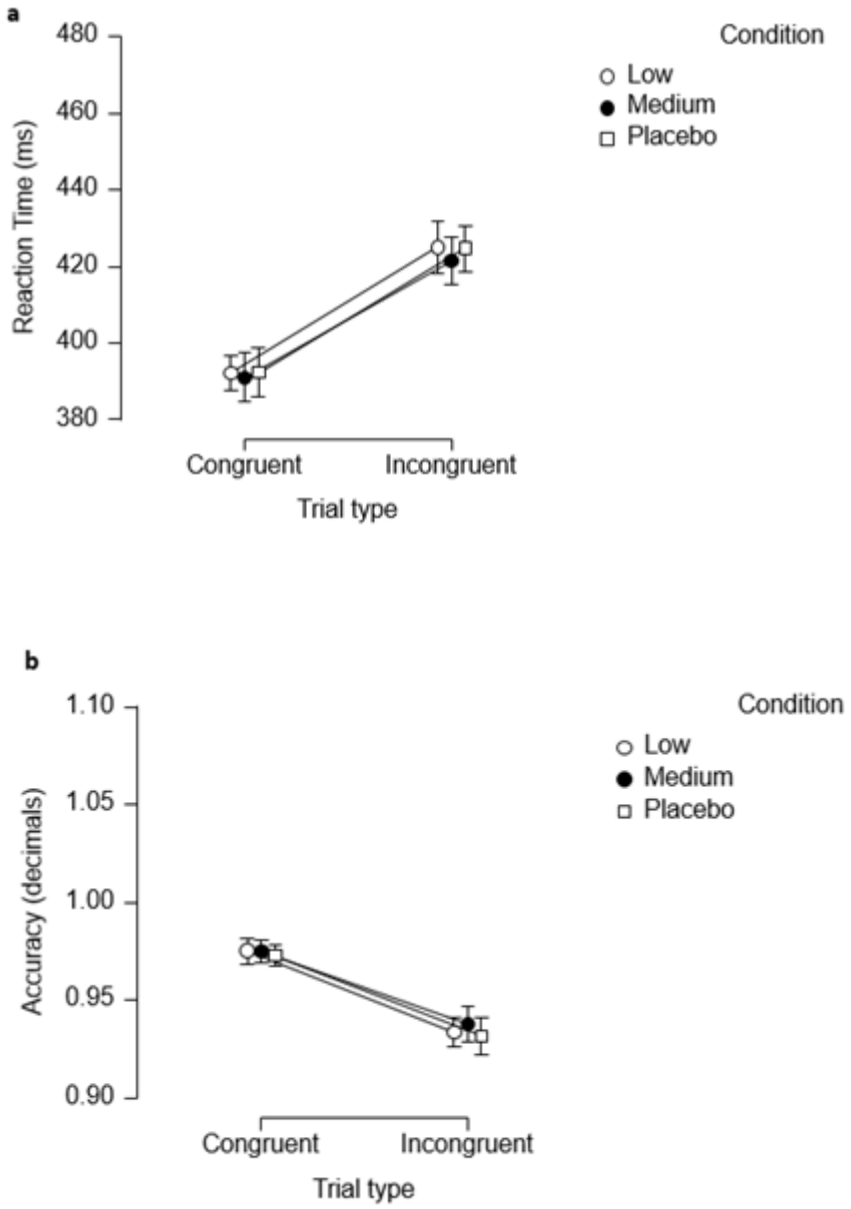


Note. Average RT (y-axis, in ms) on the Go trials are reported (a). Commission error mean (y-axis, in decimals) on the No-Go trials following CF intake is illustrated (b). The error bars represent 95% confidence intervals around the mean.

For the third hypothesis, a two-way RM-ANOVA was computed (see **Appendix C**). The main effect of CF condition on RT of the Simon task indicated a non-significant effect, $F(2,70) = 0.21$, $p = .813$, $\eta^2_p = .01$, while the main effect of trial type resulted significant, $F(1,35) = 197.67$, $p < .001$, $\eta^2_p = .85$. Further post hoc comparisons showed that participants' responded faster in congruent than incongruent trials, $M = -31.95$, $SD = 2.3$ The interaction effect analysis between CF condition and trial type indicated a non-significant effect, $F(2,70) = 0.39$, $p = .682$, $\eta^2_p = .01$. A second two-way RM-ANOVA was conducted to test CF effect on accuracy rate. Mean accuracy rate across the three CF conditions was 97.4% in congruent trials and 93.5% in incongruent trials. A significant main effect of CF condition was not found, $F(2,70) = 0.69$, $p = .503$, $\eta^2_p = .02$. The main effect of trial type was significant, $F(1,35) = 88.40$, $p < .001$, $\eta^2_p = .72$. Post hoc analysis indicated that mean accuracy rate was higher in congruent than incongruent trials, $M = 0.04$, $SD = 0.00$. Finally, the interaction between CF condition and trial type indicated a non-significant effect on accuracy enhancement, $F(2,70) = 0.35$, $p = .706$, $\eta^2_p = .01$ (see Figure 6).

Figure 6

Cognitive Results in the Simon Task



Note. Average RT (y-axis, in ms) on the Simon task are represented (a). Mean accuracy rate (y-axis, in decimals) across congruent and incongruent trials following CF intake is illustrated (b). The error bars represent 95% confidence intervals around the mean.

Table 2

Descriptive Statistics for the Flanker Task

Trial type	Condition	RT (ms)		Accuracy (%)	
		M	***SD***	***M***	***SD***
Congruent	Medium CF	394.0	33.5	98.9	1.5
	Low CF	398.1	33.1	98.6	1.3
	Placebo	400.0	32.2	98.5	1.2
Incongruent	Medium CF	431.0	38.6	95.0	3.7
	Low CF	433.5	38.3	95.4	3.6
	Placebo	434.3	34.0	95.4	3.6

Table 3

Descriptive Statistics for the Go/No-Go Task

Condition	RT (ms)		Commission error (%)	
	M	***SD***	***M***	***SD***
Medium CF	406.2	38.1	15	1.2
Low CF	400.3	44.3	16	1.3
Placebo	407.4	54.2	16	1.7

Table 4

Descriptive Statistics for the Simon Task

Trial type	Condition	RT (ms)		Accuracy (%)	
		M	***SD***	***M***	***SD***
Congruent	Medium CF	391.0	29.4	97.5	1.8
	Low CF	392.0	33.6	97.5	2.3
	Placebo	392.4	31.7	97.3	2.1
Incongruent	Medium CF	421.6	33.6	93.8	3.8
	Low CF	425.0	37.1	93.4	3.6
	Placebo	424.7	35.4	93.2	4.3

Discussion

The present study investigated whether acute CF intake affects cognitive control functions with a randomised, gender-balanced, double-blind, placebo-controlled, counterbalanced, crossover design. We hypothesised that CF might enhance interference and inhibitory control responses in healthy young adults. In particular, medium and low CF conditions would show a significant decrease in RT and accuracy rate improvement compared to the placebo condition.

We found that the CF effect did not occur in the Flanker task. RT did not significantly decrease nor the accuracy rate significantly improved across the trials, meaning that CF did not influence the difference in performance in congruent and incongruent trials. Next, the CF effect did not occur in the Go/No-Go task. We determined that CF intake did not significantly reduce RT across the Go trials nor CF significantly decreased commissions errors in the No-Go trials. In addition, CF effect did not occur in the Simon task nor caused a significant difference in the responses in congruent compared to incongruent trials. The results from our study suggested that there was no evidence for an acute CF intake effect on the modulation of interference and inhibitory cognitive responses. We found that, compared to the placebo, medium and low CF conditions did not determine a significant decrease in RT or augmented accuracy rate in the Flanker task or the Go/No-Go task or the Simon task. Therefore, we could not reject the first, second and third null hypotheses.

We could not replicate the findings from Decroix et al. (2019) since our predictions were not significant: the CF intake did not determine shorter RT in neither congruent or incongruent trials of the Flanker task. The present results are in line with previous research on executive functions (Pase et al., 2013; Decroix et al., 2016) and visual working memory maintenance and updating mechanisms (Altinok et al., 2022). Research that reported partial acute CF effects on spatial and temporal attention was conducted by Karabay et al. (2018). In

this study, RT on a visual search task was significantly decreased after CF consumption compared to placebo. However, the assessment of temporal attention with a RSVP task revealed that participants' performances were unaffected by CF supplementation. Previously, Field et al. (2011) demonstrated a significant improvement in visual processing and spatial working memory functions in the CF dose condition compared to the placebo. Scholey et al. (2011) showed limited enhancement on a RSVP task within a CDB for the high CF condition. Indeed, the improvements involved RT only and were registered in two out of six cycles of CDB.

In our study, participants scored above 93% in all three CF conditions in the Flanker and Simon tasks. That is, a ceiling effect might have appeared and it was not possible to distinguish differences in accuracy performances among participants in the medium and low CF conditions compared to the placebo. In line with our study, Karabay et al. (2018) found a similar ceiling effect in the accuracy rate in their visual search task. Moreover, previous studies (Altinok et al., 2022; Field et al., 2011; Scholey et al., 2011) did not find significant CF intake facilitation effects on cognitive task accuracy.

Similarly, research on chronic CF supplementation presents mixed evidence. For instance, Francis et al. (2006) investigated cognitive and physiological changes in participants' after they consumed CF for five days. Although no behavioural effects were found, the BOLD response significantly improved. Crews et al. (2008) found no significant difference in cognitive performance in middle-age (>60) healthy participants. Within the same participants' age range, Camfield et al. (2012) observed that there was no significant effect on a spatial working memory task. In contrast, Desideri et al. (2012) and Mastroiacovo et al. (2015) showed positive outcomes following regular CF consumption in a population of elderly people, which improved processing speed and memory-related functions. Given the

state of the art, the findings seem to corroborate the possibility that CF effect mechanisms are modulated by the participants' age.

As general strengths and limitations of the current study we considered different factors. Strengths of the present study comprehend the use of gender-balanced to not exclude gender-related differences, a double-blind study design as well as the implementation of a fast after the experimental drinks' consumption and prior to the execution of the cognitive tasks to avoid a postprandial effect in the participants. Furthermore, we evaluated whether acute CF intake influences interference and inhibitory responses using the Go-No Go and Simon tasks, which was missing in the literature. As a general limitation of our study we considered the socio-demographic characteristics of the participants. They were all young, healthy and highly educated. Indeed, they were university students and the acute CF effect might be insufficient to determine cognitive capabilities improvement. The participants' recruitment through the University platform might have increased the possibility of committing selection bias and led to a lack of representation of the population.

There could be some suggestions for future directions in this research area. The integrated use of physiological measures with cognitive tasks would be an interesting idea to apply in order to obtain a better overview about acute CF supplementation effects on participants'. Francis et al. (2006) and Decroix et al. (2019) investigated what physiological and cognitive processes were determined by CF intake. Both studies implemented fMRI and revealed a significant increment in participants' BOLD responses following CF consumption. Thereafter, it could be evaluable to integrate the same experimental design with the assessment of the Flanker, Go/No-Go and Simon tasks. Regarding participants' age, it might be an option to investigate changes in inhibitory and interference responses in a sample of

elderly participants. In this case, there could be room for improvement as the achievement of optimal cognitive functions is not guaranteed.

In conclusion, the results from the present study with a randomised, gender-balanced, double-blind, placebo-controlled, counterbalanced, crossover design suggest there is no evidence that acute CF consumption effects enhance interference and inhibitory control responses. The evidence is applicable to a sample of young and healthy adults. On the one hand, this current study did not confirm previous findings about acute CF supplementation facilitation effect on the Flanker task (Decroix et al., 2019). On the other hand, the present study implemented for the first time the Go/No-Go and Simon tasks to investigate whether CF modulates interference and inhibitory control processes and found no significant evidence. Further studies might consider these findings to examine the topic exhaustively.

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Appendix A

Statistical analysis syntax of the Flanker task

```
jaspDescriptives::Descriptives( version = "0.17.2",
        formula =  ~ congruent_A + congruent_B + congruent_C + incongruent_A + incongruent_B + incongruent_C,
        chartType = "_1noCharts",
        chartValues = "_1frequencies",
        frequencyTables = TRUE,
        qqPlot = TRUE)

jaspAnova::AnovaRepeatedMeasures( version = "0.17",
        contrasts = list(list(contrast = "none", variable = "Condition"), list(contrast = "none", variable = "Trial type"), list(contrast = "none", variable = list("Condition", "Trial type"))),
        descriptivePlotErrorBar = TRUE,
        descriptivePlotErrorBarPooled = TRUE,
        descriptivePlotHorizontalAxis = "Trial type",
        descriptivePlotSeparateLines = "Condition",
        descriptivePlotYAxisLabel = "Accuracy (decimals)",
        effectSizeEstimates = TRUE,
        effectSizeEtaSquared = FALSE,
```

```
    effectSizePartialEtaSquared = TRUE,
    plotWidthDescriptivesPlotLegend = 450,
    postHocCi = TRUE,
    postHocCorrectionHolm = FALSE,
    postHocCorrectionTukey = TRUE,
    postHocEffectSize = TRUE,
    postHocPooledError = FALSE,
    postHocTerms = list("Trial type"),
    repeatedMeasuresCells = list("congruent_A", "incongruent_A", "congruent_B",
"incongruent_B", "congruent_C", "incongruent_C"),
    repeatedMeasuresFactors = list(list(levels = list("Low", "Medium", "Placebo"),
name = "Condition"), list(levels = list("Congruent", "Incongruent"), name = "Trial type")),
    restrictedInterceptInclusion = TRUE,
    restrictedModelComparisonReference = "Complement",
    restrictedModels = list(list(informedHypothesisTest = FALSE, marginalMean =
NULL, name = "Model 1", summary = FALSE, syntax = "")),
    sphericityTests = TRUE,
    withinModelTerms = list("Condition", "Trial type", list("Condition", "Trial
type")))
```

```
jaspDescriptives::Descriptives( version = "0.17.2",
        formula =  ~ congruent_A + congruent_B + congruent_C + incongruent_A +
incongruent_B + incongruent_C,
        chartValues = "_1frequencies",
        frequencyTables = TRUE,
        qqPlot = TRUE)


jaspAnova::AnovaRepeatedMeasures( version = "0.17",
        contrasts = list(list(contrast = "none", variable = "Condition"), list(contrast =
"none", variable = "Trial type"), list(contrast = "none", variable = list("Condition", "Trial
type"))),
        descriptivePlotErrorBar = TRUE,
        descriptivePlotHorizontalAxis = "Trial type",
        descriptivePlotSeparateLines = "Condition",
        descriptivePlotYAxisLabel = "Reaction Time (ms)",
        effectSizeEstimates = TRUE,
        effectSizeEtaSquared = FALSE,
        effectSizePartialEtaSquared = TRUE,
```

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                plotWidthDescriptivesPlotLegend = 450,

               postHocCi = TRUE,

                postHocCorrectionHolm = FALSE,

                postHocCorrectionTukey = TRUE,

                postHocTerms = list("Trial type"),

                repeatedMeasuresCells = list("congruent_A", "incongruent_A", "congruent_B",
"incongruent_B", "congruent_C", "incongruent_C"),

                repeatedMeasuresFactors = list(list(levels = list("Low", "Medium", "Placebo"),
name = "Condition"), list(levels = list("Congruent", "Incongruent"), name = "Trial type")),

                restrictedInterceptInclusion = TRUE,

                restrictedModelComparisonReference = "Complement",

                restrictedModels = list(list(informedHypothesisTest = FALSE, marginalMean =
NULL, name = "Model 1", summary = FALSE, syntax = "")),

                sphericityTests = TRUE,

               withinModelTerms = list("Condition", "Trial type", list("Condition", "Trial
type")))
```

Appendix B

Statistical analysis syntax of the Go/No-Go task

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jaspDescriptives::Descriptives(
        version = "0.17.2",
        formula =  ~ NoGo_A + NoGo_B + NoGo_C,
        chartType = "_1noCharts",
        qqPlot = TRUE)


jaspAnova::AnovaRepeatedMeasures(
        version = "0.17",
        contrasts = list(list(contrast = "none", variable = "Condition")),
        descriptivePlotErrorBar = TRUE,
        descriptivePlotHorizontalAxis = "Condition",
        descriptivePlotYAxisLabel = "Accuracy (decimals)",
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        effectSizeEtaSquared = FALSE,
        effectSizePartialEtaSquared = TRUE,
        plotWidthDescriptivesPlotLegend = 450,
        repeatedMeasuresCells = list("NoGo_A", "NoGo_B", "NoGo_C"),
```

```
        repeatedMeasuresFactors = list(list(levels = list("Low", "Medium", "Placebo"),
name = "Condition")),
        restrictedInterceptInclusion = TRUE,
        restrictedModelComparisonReference = "Complement",
        restrictedModels = list(list(informedHypothesisTest = FALSE, marginalMean =
NULL, name = "Model 1", summary = FALSE, syntax = "")),
        sphericityTests = TRUE,
        withinModelTerms = list("Condition"))


    jaspDescriptives::Descriptives(
        version = "0.17.2",
        formula =  ~ Go_A + Go_B + Go_C,
        chartType = "_1noCharts",
        chartValues = "_1frequencies",
        frequencyTables = TRUE)


    jaspAnova::AnovaRepeatedMeasures(
        version = "0.17",
        contrasts = list(list(contrast = "none", variable = "Condition")),
        descriptivePlotErrorBar = TRUE,
```

descriptivePlotHorizontalAxis = "Condition",

descriptivePlotYAxisLabel = "Reaction Time (ms)",

effectSizeEstimates = TRUE,

effectSizeEtaSquared = FALSE,

effectSizePartialEtaSquared = TRUE,

plotWidthDescriptivesPlotLegend = 450,

repeatedMeasuresCells = list("Go_A", "Go_B", "Go_C"),

repeatedMeasuresFactors = list(list(levels = list("Low ", "Medium ", "Placebo"), name = "Condition")),

restrictedInterceptInclusion = TRUE,

restrictedModelComparisonReference = "Complement",

restrictedModels = list(list(informedHypothesisTest = FALSE, marginalMean = NULL, name = "Model 1", summary = FALSE, syntax = "")),

withinModelTerms = list("Condition"))

Appendix C

Statistical analysis syntax of the Simon task

jaspDescriptives::Descriptives(version = "0.17.2",

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```
        chartValues = "_1frequencies",
        frequencyTables = TRUE,
        qqPlot = TRUE)


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"none", variable = "Trial type"), list(contrast = "none", variable = list("Condition", "Trial
type"))),
        descriptivePlotErrorBar = TRUE,
        descriptivePlotHorizontalAxis = "Trial type",
        descriptivePlotSeparateLines = "Condition",
        descriptivePlotYAxisLabel = "Accuracy (decimals)",
        effectSizeEstimates = TRUE,
        effectSizeEtaSquared = FALSE,
        effectSizePartialEtaSquared = TRUE,
        marginalMeanTerms = list("Trial type"),
        plotWidthDescriptivesPlotLegend = 450,
        postHocCorrectionHolm = FALSE,
        postHocCorrectionTukey = TRUE,
        postHocEffectSize = TRUE,
        postHocPooledError = FALSE,
        postHocTerms = list("Trial type"),
        repeatedMeasuresCells = list("congruent_A", "incongruent_A", "congruent_B",
"incongruent_B", "congruent_C", "incongruent_C"),
```

```
        repeatedMeasuresFactors = list(list(levels = list("Low", "Medium", "Placebo"),
name = "Condition"), list(levels = list("Congruent", "Incongruent"), name = "Trial type")),
        restrictedInterceptInclusion = TRUE,
        restrictedModelComparisonReference = "Complement",
        restrictedModels = list(list(informedHypothesisTest = FALSE, marginalMean =
NULL, name = "Model 1", summary = FALSE, syntax = "")),
        sphericityTests = TRUE,
        withinModelTerms = list("Condition", "Trial type", list("Condition", "Trial
type")))


    jaspDescriptives::Descriptives( version = "0.17.2",
        formula =  ~ congruent_A + congruent_B + congruent_C + incongruent_A +
incongruent_B + incongruent_C,
        chartType = "_1noCharts",
        frequencyTables = TRUE,
        qqPlot = TRUE)


    jaspAnova::AnovaRepeatedMeasures( version = "0.17",
        contrasts = list(list(contrast = "none", variable = "Condition"), list(contrast =
"none", variable = "Trial type"), list(contrast = "none", variable = list("Condition", "Trial
type"))),
        descriptivePlotErrorBar = TRUE,
        descriptivePlotHorizontalAxis = "Trial type",
        descriptivePlotSeparateLines = "Condition",
```

```
        descriptivePlotYAxisLabel = "Reaction Time (ms)",
        effectSizeEstimates = TRUE,
        effectSizeEtaSquared = FALSE,
        effectSizePartialEtaSquared = TRUE,
        plotWidthDescriptivesPlotLegend = 450,
        postHocCorrectionHolm = FALSE,
        postHocCorrectionTukey = TRUE,
        postHocEffectSize = TRUE,
        postHocPooledError = FALSE,
        postHocTerms = list("Trial type"),
        repeatedMeasuresCells = list("congruent_A", "incongruent_A", "congruent_B",
"incongruent_B", "congruent_C", "incongruent_C"),
        repeatedMeasuresFactors = list(list(levels = list("Low", "Medium", "Placebo"),
name = "Condition"), list(levels = list("Congruent", "Incongruent"), name = "Trial type")),
        restrictedInterceptInclusion = TRUE,
        restrictedModelComparisonReference = "Complement",
        restrictedModels = list(list(informedHypothesisTest = FALSE, marginalMean =
NULL, name = "Model 1", summary = FALSE, syntax = "")),
        sphericityTests = TRUE,
        withinModelTerms = list("Condition", "Trial type", list("Condition", "Trial
type")))
```