

Does caffeine matter for arousal? Affective and autonomic responses induced by caffeine in coffee intake: evidence from a double-blind tasting task

¿Importa la cafeína para la excitación? Respuestas afectivas y autónomas inducidas por la cafeína en la ingesta de café: evidencia de una tarea de degustación doble ciego

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Abstract

Coffee is consumed worldwide, but there are different types of espresso blends, each with its unique concentration of caffeine, which can have different effects on the human being. The aim of this study was to understand the effect of the impact of caffeine on the autonomic nervous system, evaluating the physiological changes and subjective responses due to different levels of caffeine intake. A double-blind tasting task consisting of one within-subject factor design (caffeine level: high / double caffeine mixture (blend A) vs single-charge caffeine mixture (blend B) vs low-caffeine mixture (blend C) allowed us to assess participants' autonomic responses using Heart Rate Variability (HRV) and Pupillary Reactivity (PR). Arousal was also assessed through the Self-Assessment Manikin (SAM). Results revealed statistically significant differences in HRV and PR between coffee blends, showing the blend A, a more pronounced autonomic response than blend C. However, no significant differences were found in arousal level among coffee blends. These results are similar to previous research that pointed out to a discordance between subjective and objective measures when caffeine is consumed.

Keywords: Affective valence; Caffeine; Autonomic response; Pupil response; Heart rate variability.

Resumen

El café se consume en todo el mundo, pero existen diferentes tipos de mezclas de expreso, cada una con su concentración única de cafeína, que puede tener diferentes efectos en el ser humano. El objetivo de este estudio fue comprender el efecto del impacto de la cafeína en el sistema nervioso autónomo, evaluando los cambios fisiológicos y las respuestas subjetivas debido a los diferentes niveles de ingesta de cafeína. Una tarea de degustación doble ciega, que consiste en un diseño de factores intraindividuales (nivel de cafeína: mezcla de cafeína alta/doble (mezcla A) vs mezcla de cafeína de carga única (mezcla B) vs mezcla baja en cafeína (mezcla C), nos permitió evaluar las respuestas autónomas de los participantes utilizando la variabilidad de la frecuencia cardíaca (VFC) y la reactividad pupilar (RP). La excitación también se evaluó mediante el Self-Assessment Manikin (SAM). Los resultados revelaron diferencias estadísticamente significativas en la VFC y la RP entre las mezclas de café, mostrando la mezcla A respuesta autonómica más pronunciada que mezcla C. Sin embargo, no se encontraron diferencias significativas en el nivel de excitación entre las mezclas de café. Estos resultados son similares a investigaciones anteriores que señalaron una discordancia entre medidas subjetivas y objetivas en el consumo de cafeína.

Palabras clave: Valencia afectiva; Cafeína; Respuesta autonómica; Respuesta pupilar; Variabilidad de la frecuencia cardíaca.

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INTRODUCTION

Coffee consumption is a worldwide phenomenon (Hewlett & Wadsworth, 2012) and the most commonly consumed beverage in the world (Graham, 2001). The caffeine present in coffee is said to be a lipophilic molecule which easily crosses the blood-brain barrier, and increases neurotransmitter concentration in the brain, therefore is designated as a central nervous system stimulant. The average amount of caffeine consumed in the U.S. population has remained constant at approximately 300 mg per person per day (Liu & Song, 2015). The global retail coffee market has shown significant growth over the past 5 years (about 2.3% per year), having represented around USD 86.5 B and 5.9 M tonnes in 2018. From the roasted bean and ground segments, capsules represent 64% of market and presented an average annual progression of 3.1% between 2013 and 2018. This growth is more accentuated in unidoses solutions such as capsules, being this the product of greatest growth expected until 2024. This pace of growth in coffee consumption encourages companies in the sector to expand their product portfolio, through innovative solutions that provide new consumption experiences, and to spread their brands through strategies that support internationalization and market diversification. The caffeine present in coffee is said to be a lipophilic molecule which easily crosses the blood-brain barrier, and increases neurotransmitter concentration in the brain, therefore is designated as a central nervous system stimulant.

THE EFFECTS OF CAFFEINE INTAKE ON HUMANS

Caffeine's greatest effect takes place in the basal ganglia, where its inhibitory action on adenosine receptors and synergistic effect with dopamine turn off pathways which act to restrict motor activation signals in the brain. High caffeine doses induce adenosine antagonism and phosphodiesterases inhibition, interacting with the sympathetic nervous system and inducing β_1 -receptor activation. This results in positive inotropic and chronotropic effects, accountable for an augmented heart rate and conductivity (Cappelletti, 2015). In fact, higher concentrations of caffeine increase intracellular cAMP and cyclic guanosine monophosphate (cGMP) by a nonspecific phosphodiesterases inhibition, which affects cardiac contractility secondary to calcium release.

An extensive body of literature has examined the effects of caffeine on mood, which showed that increases in low doses of caffeine resulted in increases in subjectively reported positive affect (Brunyé, Mahoney, Lieberman & Taylor, 2010; Smith & Rogers, 2000). Caffeine also appears to provide significant ergogenic effects on muscle strength and power (Grgic, Trexler, Lazineca & Pedisic, 2018). Apart from mood and exercise effects, few studies on affective modulation and emotional processing when correlated with autonomic response were found in the literature. In general, after drinking coffee, consumers report feeling more energetic, imaginative, efficient, confident, alert, and focused, as well as motivated and socially active (Griffiths & Mumford, 1995). Recent meta-analysis studies showed benefits of caffeine intake in providing significant ergogenic effects on muscle strength and power (Grgic et al., 2018), significantly increase isokinetic strength

(Grgic & Pickering, 2019) enhance components of anaerobic performance (Grgic, 2018), a protective effect on the decreasing risk of depression (Grosso, Micek, Castellano, Pajak, & Galvano, 2016; Wang, Shen, Wu & Zhang, 2016), reduction for various health outcomes at three to four cups a day, like lower risk of incidence of cancers and neurological, metabolic, and liver conditions.

A meta-analysis showed significant ergogenic effects of caffeine intake on maximal muscle strength of upper body and muscle power (Grgic et al., 2018). Caffeine's pro-arrhythmic effects at high doses are supported by animal studies (Mehta, Jain, Mehta & Billie, 1997; Balasubramaniam, Chawla, Grace & Huang, 2005), which have been performed with higher doses of caffeine and evaluation by invasive techniques. Numerous physiological and epidemiological human studies have investigated the link between caffeine and both atrial and ventricular arrhythmias (Pelchovitz, Goldberger, Jeffrey, & Goldberger, 2011), but results are not always in consonance.

The acute toxic level of caffeine is not well established, but for adults it is approximately 10 g/day, which is comparable to a consumption of approximately 100 cups of a regular espresso coffee (Greden, 1974). The consumption of caffeine is also known to increase a person's cardiac minute volume and cardiac index (Corti et al., 2002; Cano-Marquinaa, Tarínb & Canoc, 2013) and can also bind directly to the vascular smooth muscle cell receptors and, through similar mechanisms, cause vasodilation (Echeverri, Montes, Cabrera, Galán & Prieto, 2010). Caffeine was shown to be capable of delaying parasympathetic recovery but did not influence the behaviour of the respiratory rate, oxygen saturation or frequency-domain HRV indices (Gonzaga, Vanderlei, Gomes & Valenti, 2017; Gonzaga, Vanderlei, Gomes, Garner & Valenti, 2019) These effects are more pronounced in irregular caffeine consumers compared to regular consumers, who show minimal effects of caffeine on the cardiovascular system (Izzo, Ghosal, Kwong, Freeman & Jaenike, 1983). Caffeine intake produces a higher rise in diastolic blood pressure than in systolic blood pressure, which may be due to the antagonistic binding of caffeine on adenosine receptors, which results in vasoconstriction (Sudano et al., 2005; Smits, Lenders & Thien, 1990). A moderate ingestion of caffeine (100 mg) reveals no decrease in digital blood flow measurement (Knight, Pagkalos, Timmons & Jose, 2015), while higher doses of caffeine can result in an accelerated heart rate, but these effects are not common in people who consume caffeine regularly. In fact, there is evidence that the habitual consumer of caffeine develops a tolerance to its cardiovascular and neuroendocrine effects (Lane, Adcock, Williams & Kuhn, 1990; Lane, Pieper, Phillips-Bute, Bryant & Kuhn, 2002).

Further, caffeine produces mild autonomic nervous system arousal and improved mood when compared to a non-caffeinated placebo (Quinlan et al., 2000; Cappelletti, 2015). Despite several research studies that addressed coffee intake and its influence on various aspects of human life, few of them were done using the minimum quantities needed for the physiological effects to be registered. One of these studies, performed by Smit & Rogers (2000), aimed to measure the effects of small doses of caffeine (0 mg, 12.5 mg, 25 mg, 50 mg, and 100 mg) on cognitive performance and mood. The authors reported that, even in small doses, caffeine consumption resulted in improved cognitive

task performance. However, individuals who consumed caffeine habitually had larger gains in cognitive performance than those who only consumed caffeine occasionally. In another study, Brunyé et al. (2010) addressed the effects of different doses of caffeine (0 mg, 100 mg, 200 mg and 400 mg) on attention, showing that caffeine consumption produced an increase in alertness and in the performance of executive tasks. The best performance was achieved with 200 mg of caffeine on one intake or more (Brunyé et al., 2010).

Caffeine can also affect the cardiovascular response to physical activity. Normally, during physical activity, both heart rate and blood pressure increase. However, regular caffeine intake has a stabilizing effect on blood pressure, which provokes a small increase in baseline values and therefore only a modest increase during physical activity (smaller than for non-coffee drinkers) (Höfer & Bättig, 1993; 1994; Grgic, 2018). Recent studies show that caffeine ingestion increases resting cardiac autonomic modulation (Sarshin et al., 2020). With or without the taste of coffee, the caffeine effects on autonomic arousal, respiratory response, and reported alertness are well known. Caffeine, after ingestion, is absorbed by the gastrointestinal tract in approximately 45 minutes, and its concentration in the blood is highest approximately one hour after ingestion. With a half-life of between two and a half hours and four and half hours, caffeine is present in the bloodstream for about six hours after ingestion (Nehlig, 2010). However, some studies indicate that the effects of the substance are felt almost immediately, only a few minutes after ingestion (Adan, Prat, Fabbri & Sánchez-Turet, 2008). One of the most common effects reported after caffeine intake is the feeling of thirst. However, regular coffee drinkers develop a tolerance to that sensation, thus not experiencing an increase in thirst when drinking coffee (Smith & Rogers, 2000). Caffeine significantly induces regulators of mitochondrial biogenesis and oxidative metabolism, suggesting that induced physiological levels of caffeine appear to enhance cell metabolism (Schnuck et al., 2018).

There is a growing interest in the use of Heart Rate Variability (HRV) (Nathelson, 1985; Marães, 2010; Benjamim et al., 2020) and pupillary response (PR) as measures of autonomic activity (Lovallo et al., 2004; Grant & Ker, 2008; Bouffard, 2019) linked to affective and autonomic processing (Bradley, Miccoli, Escrig & Lang, 2008). HRV is a non-invasive method to assess autonomic functioning of the heart from a simple electrocardiogram recording. HRV is manifested by the variability between successive heart beats (R-R intervals), which reflects the sympathetic and parasympathetic activity of the autonomic nervous system. Pupillary activity, that is, contractions and dilations of the pupils, is also thought a reliable autonomic index and it can be recorded without any attachments to the body, through an eye-tracking system (e.g., Esteves & Rosa, 2019). However, evidence from these studies has been contradictory (Wilhelm, Stuiber, Lüdtké & Wilhelm, 2014). Moreover, studies assessing the impact of caffeine on the cardiovascular, pupillary and affective systems simultaneously while tasting coffee are not common. As we know, the knowledge about the sensory and perceptual properties of caffeine has theoretical importance as well as practical implications for coffee producers. Therefore, the main goal of the present study was to gain a deeper understanding how caffeine intake

impacts on the affective and autonomic response while tasting some commercial Portuguese espresso coffee blends, in particular a blend that has enriched caffeine content (double charge). We pretend to verify whether the advertising claim that a “double shot” of caffeine has double the impact on the human body and brain is true, and whether a double shot is more satisfying for consumers.

We propose that the level of caffeine intake affects the affective system (arousal) and autonomic responses, so that higher levels of caffeine should lead to a higher level of arousal, an increased cardiac and pupillary activity (Wilhelm et al., 2014; Woo & Kim, 2015), so we formulated the following hypotheses:

1. Higher Pupillary Reactivity (dilatation) on blend A compared to other blends.
2. Higher SDNN for blend A than for other blends.
3. More pronounced sympathetic-vagal balance to blend A in comparison to the other blends.
4. Higher subjective arousal for blend A compared to the other blends.

METHODOLOGY

Participants

Our study consisted of a convenience sample of 20 volunteers. Of these, 60% were women ($n = 12$) and 40% were men ($n = 8$). The mean age was 40.41 years old ($SD = 9.18$), ranging between 22 and 56 years. Only one participant was not Portuguese (1.7%, Russian) but he has been living in Portugal for several years. With regard to the coffee consumption habits, all participants reported being regular consumers who have no brand preferences and who do not have a capsule machine for making coffee. On average, participants referred to consume 3.1 coffees ($SD = 1.29$) per day and they have been consuming coffee for an average of 22.26 ($SD = 8.31$) years. Concerning the control of caffeine intake before our task, participants referred they had not drunk coffee for an average duration of 5.9 hours ($SD = 6.81$). All participants were Portuguese and reported normal medical history with no hearing or visual problems. Participants were treated in accordance with the American Psychological Association’s ethical code (APA, 2010).

Instruments and measures

A short Google Docs form was created in order to collect socio-demographic data (nationality, gender, age, occupation) as well as information regarding to the participants’ coffee-drinking habits. The arousal, as subjective affective dimension, was assessed by using the Self Manikin Assessment (SAM; Bradley & Lang, 1994). The SAM is a pictorial measure that assesses affective responses to stimuli in the dimensions of pleasure and arousal (pleasure was not evaluated in this study) (Figure 1).

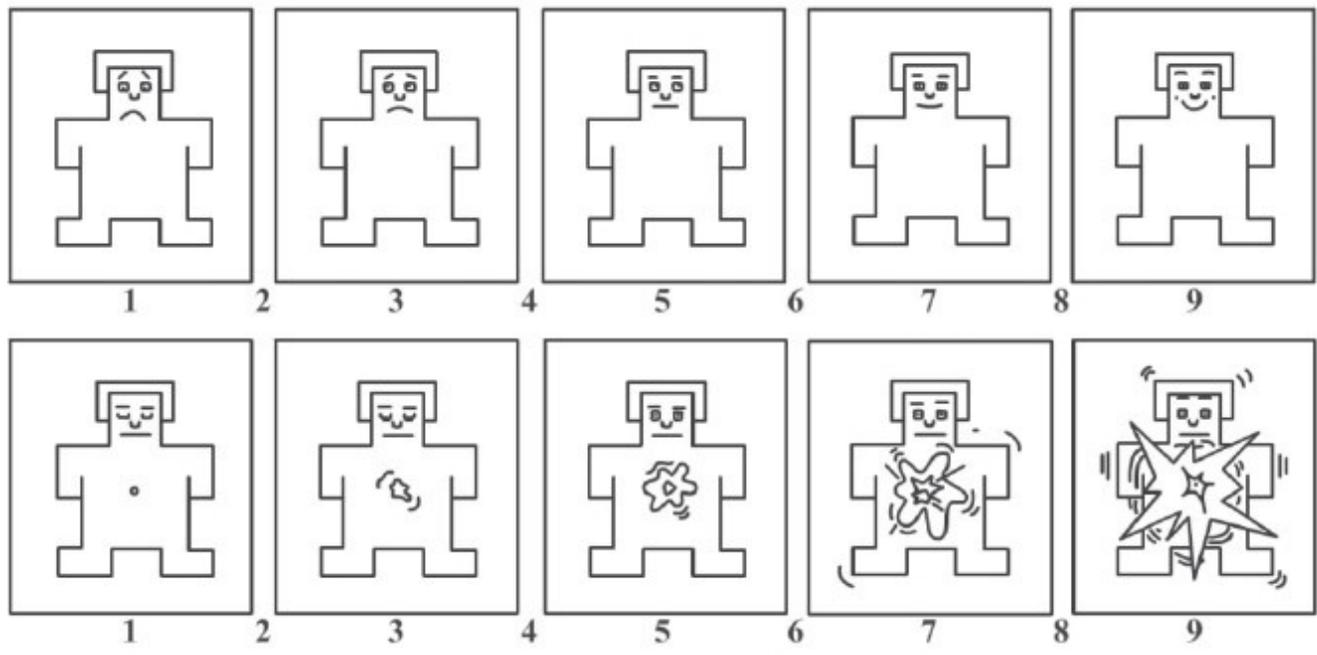


Figure 1. The Self-Assessment Manikin (SAM) measures of pleasure (top panel) and arousal (bottom panel)
Source: Authors.

Autonomic physiological indices

Two physiological indices, namely the Heart Rate Variability (HRV) and Pupillary Reactivity (PR) were examined in order to objectively assess the impact of caffeine intake on the autonomic system during the double-blind tasting task. The literature suggests HRV and PR to complement the limitation of the SAM because they reflect the state of autonomic nervous system (Carvalho & Rosa, 2020; Rosa et al., 2020).

Experimental Procedure and Apparatus

The study was conducted in one session at the neurosensory laboratory of the CATA-A (Castelo Branco – Portugal) as follows: 1) reception and general information was given to participants, 2) completion of the consent form, 3) assembly of electrodes for ECG recording, 4) eye tracker calibration; 5) the random assignment to different experimental conditions, that is, a different tasting order. Participants were instructed to remain as still as possible and to look at the eye tracker monitor throughout all the phases within the task. The SAM was applied three minutes after the consumption of each coffee blend. All blends were in espresso capsule form, dispensed by calibrated machines. Three commercial Portuguese coffee blends, particularly a blend that has enriched caffeine content (double charge) and referred to as blend A, a blend B was a normal blend from the same brand company and blend C was the direct equivalent to blend B, but from the main competing brand/company in the Portuguese market. The characteristics of the examined blends are shown at Table 1.

TABLE 1.
 Caffeine level for each coffee blend.

Coffee Blend	Caffeine (mg/100ml)	Caffeine for Espresso Cup (mg/35ml)
Blend A	414,34	145,02
Blend B	281,42	98,50
Blend C	202,94	71,03

Source: Authors.

The double-blind tasting task was conducted by two technicians who had no knowledge about the study main goal. The dispensing of coffee was performed in accordance with the established technical standards. After drinking each coffee blend, participants performed a blind tasting evaluating the arousal-eliciting capacity of the tasted stimuli. They tasted 35 ml of each coffee blend and rated their arousal (one question: “How much aroused you were after drinking this coffee blend?” using a nine-point Likert scale. At the end, participants were thanked and dismissed. The participants took an average of 20-25 minutes to complete the task. The participation activity sequence is shown in Figure 2.

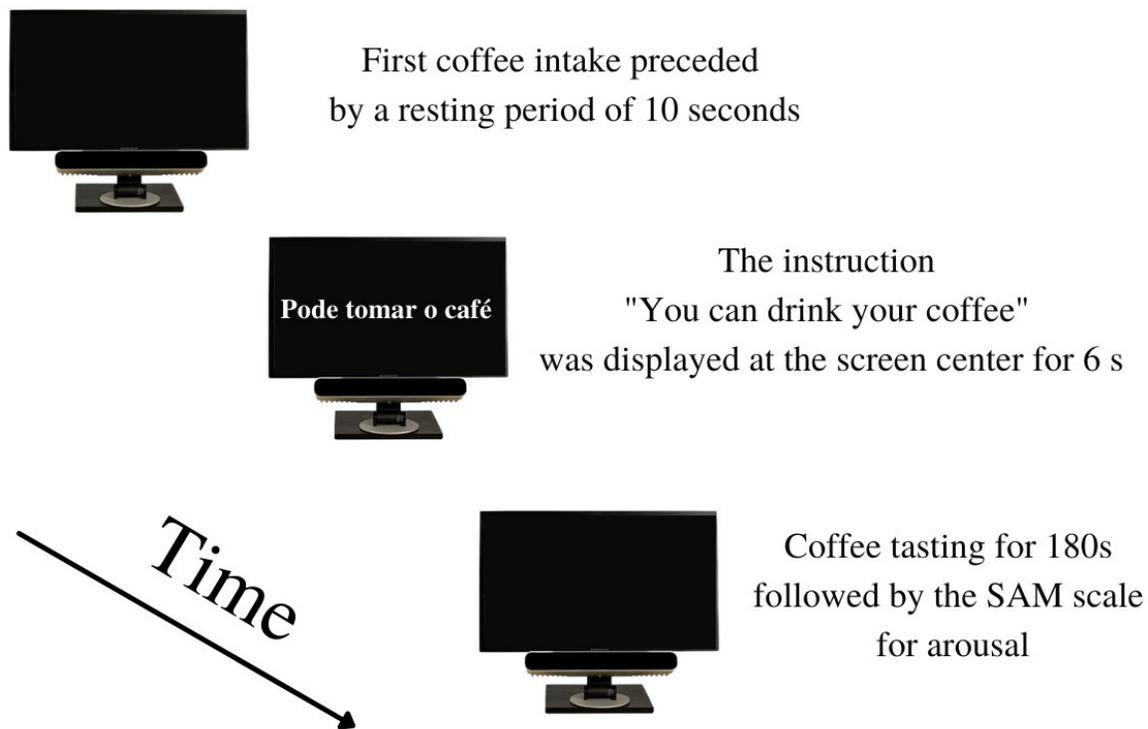


Figure 2. The sequence of the experimental task.
 Source: Authors.

Cardiac activity was performed using the plugged version of the BITalino (PLUX Wireless Biosignals, 2020). The BITalino is a wireless realtime biosignals acquisition unit with multimodal sensors (Figure 3).



Figure 3. BITalino Acquisition Unit (plugged version).
Source: Authors.

The measurement of cardiac activity was performed using a bipolar montage, using three clip-in disposable electrodes (+, -, ground) with a sodium chloride (NaCl) based electrolytic paste. These electrodes were placed on the chest area near the heart, forming the Einthoven triangle. The electrocardiogram was recorded at 1000 Hz with the [Open Signals recording software \(Plux\)](#) shown in [Figure 4](#). A manual trigger was sent via a light (lux) sensor from BITalino.

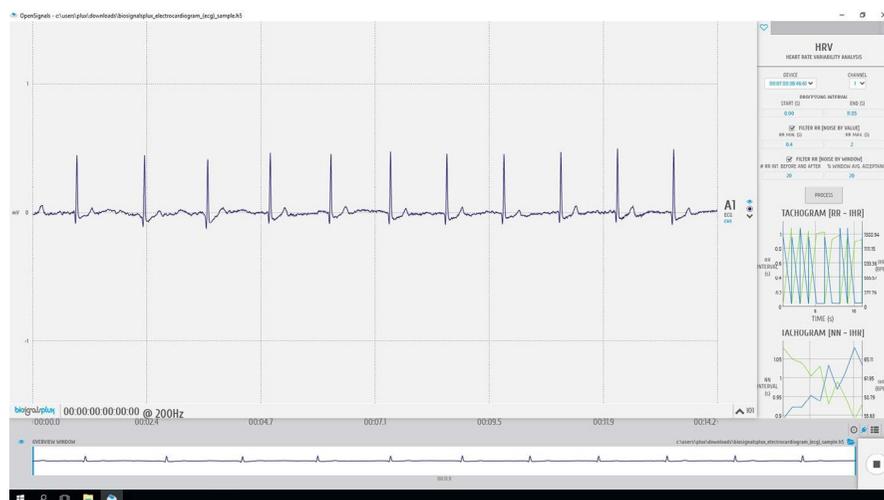


Figure 4. Cardiac activity recorded via Opensignals software.
Source: Authors.

Pupillary activity was continuously recorded at a 500 Hz sampling rate with an average accuracy of 0.5° of visual angle through the SMI (Sensometric Instruments, GmbH, Germany) RED500 eye-tracking system. All instructions were presented visually via the SMI Experiment Suite 360, a stimuli presentation program that is packaged with the SMI (Sensometric Instruments) RED500 eye-tracking system. This system, connected to a 22" LCD monitor and a Dell Intel Core2Duo laptop computer. A specific trigger was automatically built via light sensor at the beginning of each event (three in total). This allowed the computation of the baseline for both autonomic indices. Eye tracking calibration was performed using a 9-point system (Rosa et al., 2016). Participants were instructed to keep still in order to keep a distance of 60 cm from the centre of the screen during the task (e.g., Rosa et al., 2015; Rosa, 2017; Rosa, Castrillón, Castillo, Piedrahita & Díaz, 2018).

Data reduction and statistical analysis

Only 10% of the electrocardiograms ($n = 2$) presented excessive noise and therefore, were excluded from the analysis. Due to differences in sampling rate, PR and HRV data were analyzed separately, thus avoiding up-sampling and/or down-sampling of either measure. Eye blinks, ocular deviations, and outliers (± 3 SD) were removed from the pupillary activity raw data and linearly interpolated for each trial (Rosa, Esteves & Arriaga, 2015). Pupil artifacts were randomly distributed across experimental conditions. Pupil data was converted from pixels to millimeters and then exported individually to the software AcqKnowledge (v. 4.1). Pupil data was smoothed with a digital filter (FIR) low-pass 4Hz (Hamming windowing) with 500 coefficients. PR was evaluated based on the pupil dilation ratio. This ratio was calculated by dividing the maximum value of pupil size, measured during 180s after caffeine intake, by the maximum value of pupil diameter, measured 5s before caffeine intake. To examine the effects of caffeine intake on PR, the 180s period was subdivided into three segments of 60 seconds (Paschoal, Petrelluzzi & Gonçalves, 2002). Before spectral analysis for HRV, artifacts were detected, identified, and excluded from the analysis. Frequency bands (Very Low Frequency 0 Hz – 0.04 Hz; Low Frequency 0.04 Hz - 0.15 Hz; High Frequency 0.15 Hz - 0.4 Hz; Very High Frequency 0.4 Hz - 3.0 Hz) were analysed. The Power Spectral Density (PSD) was obtained using Fourier Fast Transform (FFT) with a Hamming windowing and a linear filter. The sympathetic-vagal balance was calculated automatically by the standard formula (LF/HF). All autonomic indices were analyzed through the software Acknowledge (v. 4.1).

All of the statistical analysis was done using the IBM SPSS Statistics (v. 20.0) for Windows. Parametric tests were applied due to its robustness to violation of the normality assumption (Marôco, 2010). The Pearson-Bravais correlation coefficient was performed to analyse linear relations between variables. Univariate analysis was conducted using t-tests and one-way ANOVAs. Multivariate analyses were performed using ANOVA for repeated and mixed measures. The Greenhouse-Geisser correction was used to report significant results. The Bonferroni correction was applied for multiple comparisons of means. All statistical tests were performed for a significance level of 0.05 (Stevens, 1992).

RESULTS

Assessment of potential confounders

In order to identify potential confounders, the coffee consumption habits between male and female volunteers were compared. Results revealed no statistical differences for coffee consumption between males and females (Table 2).

TABLE 2.
Mean, standard deviation and respective significance tests for caffeine intake indicators by gender.

	Male (n = 8)		Female (n = 12)		t
	M	SD	M	SD	
Average daily coffee intake.	3.33	1.44	2.75	1.04	-0.987
Years of consumption.	21.25	6.89	24.00	10.71	-0.685
How many hours ago did you have your last coffee?	5.58	6.75	6.38	7.50	-0.246

Source: Authors.

Pupillary reactivity (PR)

The first step of our analysis was to examine whether the pupil diameter at baseline did not differ among coffee blends. Two repeated-measures ANOVAs showed neither significant difference in mean pupil size [$F(2,38) = 0.434$, $p = 0.620$] nor in maximum pupil size [$F(2,38) = 1.51$, $p = 0.233$] at baseline. Subsequently, a repeated-measures ANOVA showed no significant differences in PR between coffee blends [$F(2,38) = 1.23$, $p = 0.304$]. To examine potential differences in PR between the three distinct moments (0s-60s, 60s-120s; 120s-180s) during caffeine intake, a 2-way repeated measures ANOVA [3 (blends) \times 3 (moments)] was performed.

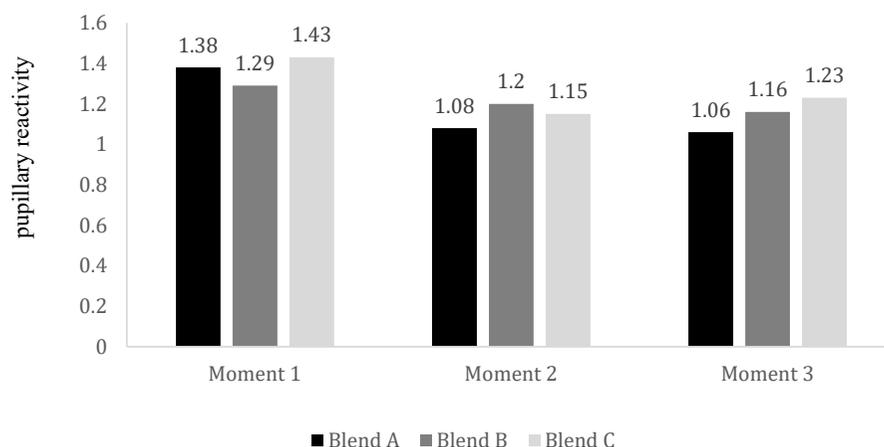


Figure 5. Pupillary reactivity (PR) as a function of coffee blend and moment (0s-60s; 60s-120s; 120s-180s).

Source: Authors.

Results showed a significant effect for the different moments [$F(2.38) = 17.55$, $p < 0.001$], with a more pronounced PR at the first moment ($M = 1.37$) than at the second ($M = 1.14$) and at the third ($M = 1.15$) moments, independently of the coffee blend. However, there were no differences between the second and third moments. There were neither significant main effects nor interaction effects (all p s > 0.05) (Figure 5).

Heart rate variability (HRV)

As for pupil dilation, it was examined whether the participants had similar SDNN baseline values for the three coffee blends. Results indicated no significant differences in SDNN between the at baseline for the three blends $F(2.38) = 1.66$, $p = 0.210$. Right after, the SDNN ratio (SDNN during the caffeine intake (180s - before caffeine intake SDNN)) was computed for each moment, and a mixed ANOVA was performed. Results showed significant differences in SDNN ratio between coffee blends [$F(2.17) = 7.190$, $p = 0.005$], presenting the blend A a significant higher SDNN ($M = 0.01$) than blend B ($M = -0.07$), but not than blend C ($M = -0.001$) (see Figure 6). There were no other significant main or interaction effects.

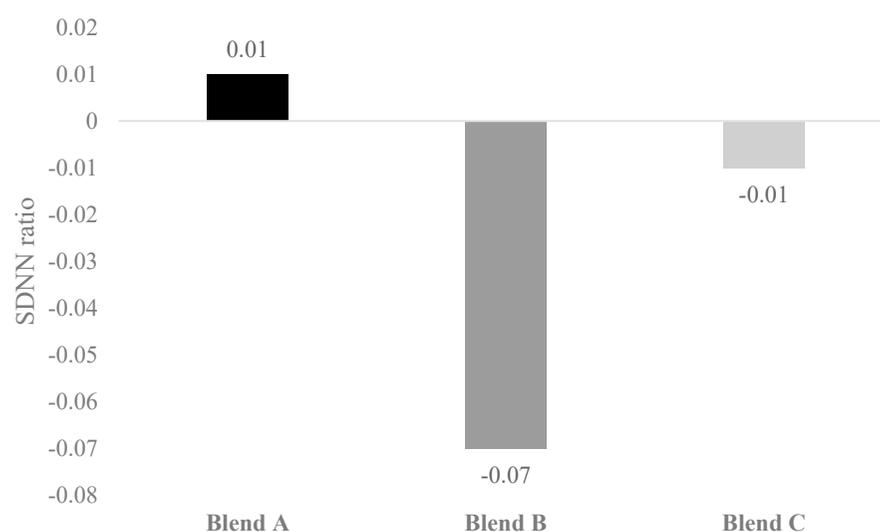


Figure 6. SDNN Ratio across blends.
 Source: Authors.

In order to analyse the sympathetic-vagal balance, the HRV index (i.e., low-frequency divided by the High-Frequency power (LF / HF)) was computed. The repeated measures ANOVA showed significant differences in the sympathetic-vagal balance between the three coffee blends [$F(2.38) = 19.700$, $p < 0.001$] as shown in Figure 7.

Blend A showed significantly less sympathetic-vagal balance ($M = 1.60$) than blend C ($M = 6.77$). However, there were no significant differences between blend A and blend B.

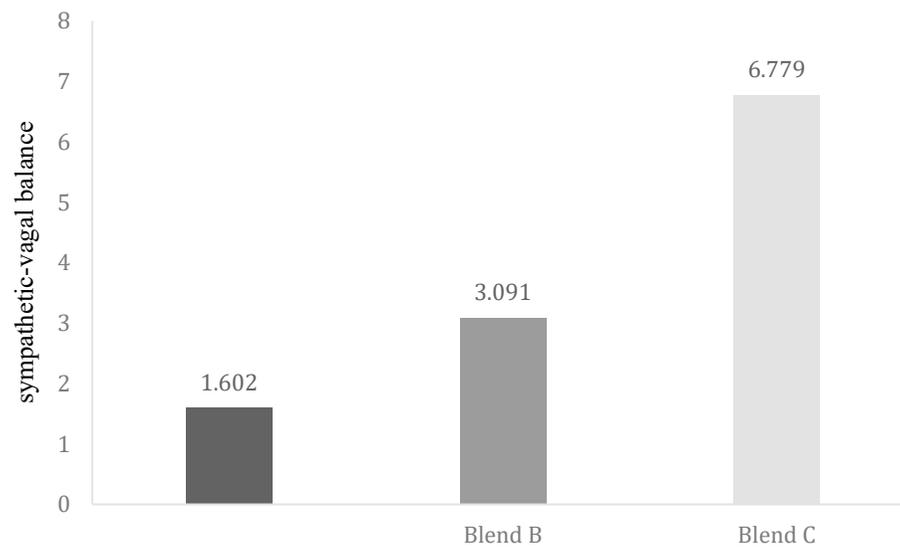


Figure 7. Sympathetic-vagal balance between coffee blends.
Source: Authors.

Subjective arousal

A repeated measures ANOVA was conducted in order to examine whether subjective arousal was different between coffee blends. Results showed marginally significant differences [$F(2,36) = 2.62$, $p = 0.087$] between the three blends. A tended to be perceived as more stimulating/arousing ($M = 6.31$) than blend C ($M = 5.31$), but not than blend B ($M = 6.26$) (Figure 8).

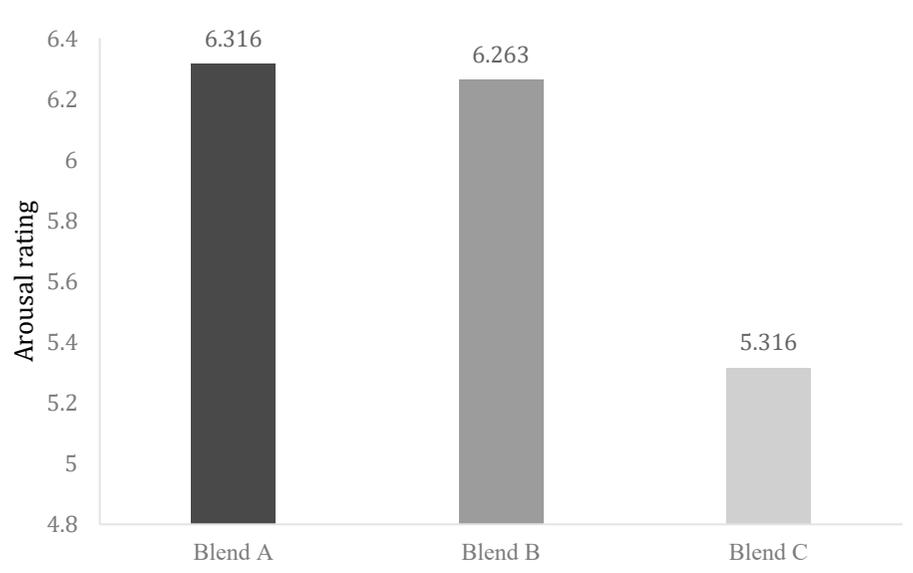


Figure 8. Subjective arousal between coffee blends.
Source: Authors.

DISCUSSION

The present study intended to investigate how coffee blends with different caffeine level impact on the autonomic and affective system. Results partially support the first hypothesis, indicating that different levels of caffeine can influence the autonomic nervous system (Koenig et al., 2013), namely the pupillary system. Caffeine, through the activation of

noradrenergic nerves, can trigger sympathetic stimulation that results in changes in PR (Nehlig, Daval & Debry, 1992). However, contrary to our expectations, it was not observed a higher PR for blend A when compared to other blends at the first moment of the tasting task, but for blend C. This could be explained for the differences in the smell/aroma of the espresso blend has explained before that is extremely important (Samoggia & Riedel, 2019). Still, a higher PR in blend A was found at the 2nd moment. This may represent an additive effect when drinking two consecutive blends, particularly when blend B precedes blend A whose caffeine volume of the 2 blends exceeds 200mg causing reactions in the body in usual coffee consumers as previously suggested (Brunyé et al., 2010). Participants who consumed the coffee blend combination with the highest level of caffeine (blend B + blend A) showed higher PR. Our results are in concordance with studies that have shown that higher autonomic activity induced by caffeine intake led to a higher pupillary activity, specifically a larger pupil dilation (Steinhauer, Siegle, Condray & Pless, 2004; Bouffard, 2019). The small effects - we have found might be due to the sensitivity of the pupillary system to room brightness as well as to cognitive load which were not controlled in this study (Beatty & Lucero-Wagoner, 2000; Rosa, Caires, Costa, Rodelo & Pinto, 2014; Partala & Surakka, 2003).

Regarding the cardiac activity, our results partially confirmed the second/third hypotheses. There were significant differences between HRV, particularly a higher SDNN (parasympathetic activation indicator), showing the blend A, a positive SDNN variation (SDNN coffee tasking - SDNN baseline) in contrast to blend B and C, both with SDNN variation below 0. These results are in line with other studies of experimental nature (Hibino, Moritani, Kawada & Fushiki, 1997; Richardson et al., 2009; Cappelletti, 2015; Grgic et al., 2018). The results of the sympathetic-vagal balance are congruent with the SDNN, as the high frequency power is part of the denominator of the LF/HF ratio. These results are also in line with the work of Syce, Veliath and Krishnamurthy (2014). In a similar study, Hibino et al. (1997), observed that there was a significant increase in high-frequency power after ingestion of 240 mg of caffeine, that only happens on the second intake of blend A after intake blend B (M = 243.52 mg of caffeine intake). The results of this study are supported by recent research that has shown that caffeine increases cardiac vagal activity in healthy middle-aged people (Monda et al., 2009). SDNN was significantly higher in blend A when compared to blend B, indicating a recovery of the parasympathetic activity, stimulated by sympathetic-vagal balance. However, some contradictory results were obtained in certain studies, where no significant difference in SDNN nor in RMSSD between situations with different levels of autonomic activation (Tharion, Parthasarathy & Neelakantan, 2009; Melillo, Bracale & Pecchia, 2011). The concept of “sympathetic-vagal balance” reflects the autonomic state resulting from the sympathetic and parasympathetic influences, considered has being the ratio between LF and respiratory-frequency powers. Sympathovagal balance is simply the ratio of absolute LF to absolute HF power, or LF/HF (Goldberger, 1999). Reduced parasympathetic activity occurs frequently in response to drug therapy. In some cases, the drug is specifically intended to inhibit the parasympathetic system, but many drugs commonly used for other purposes also have peripheral antimuscarinic effects. At very high doses, gastric emptying and gastric secretion may also be inhibited, leading to epigastric discomfort (Tonkin, 2009). We found there was also a significant change

in the sympathetic to parasympathetic activity represented by the LF/HF ratio, that can reflect for one side this trend to caffeine can be, at some dosage, considered as stimulant and this means that higher levels of caffeine, lower sympathetic-vagal balance.

As research has shown that caffeine intake increases mental energy and induces greater alertness (e.g., Bruce, Scott, Lader & Marks, 1986; Lanini, Fernandes & Pompéia, 2016) it was expected that this would be reflected in subjective arousal. Although blend A produced higher levels of arousal, these were not statistically significant, which not corroborate our fourth hypothesis. These results are similar to a study by Ahmadi, Mokhtari, Kazem and Mousavi (2012), which found no differences in arousal associated with caffeine intake. This study is also consistent with other studies that found a discordance between subjective and objective measures (e.g., Chivers, Gerulf, Latty & Bailey, 2004; Rosa et al., 2014, 2017).

The results in general support the idea that blend A is different in terms of the physiological responses that it induces, and when taken after blend B (aprox. 250 mg caffeine intake), supports the vision of Brunyé et al. (2010) that after 200mg caffeine intake reached the excitatory autonomic response. Nevertheless, these results need to be interpreted with caution given the experimental task design. One limitation is related to the brief time interval between blends that might create a carryover effect so the last tasted blends would reflect the autonomic effects from the previous blends. Finally, a potential caveat is that results are based on a small sample size that may increase Type II error in the statistical analyses. Future studies with larger samples would certainly give more statistical power to the results if possible, examine potential autonomic and affective differences across gender.

CONCLUSIONS

In conclusion, our results suggest that cardiac and pupillary activity are modulated by caffeine levels. We also found that affective experiences can be partially dissociated from autonomic activations (Rosa et al., 2014), which support the use of implicit measures to study consumer preferences of food and beverage in the future (Rodrigues, 2015). The findings of this study offer valuable insights into the relationship between caffeine intake and affective and autonomic responses. These results could suggest that coffee blends with higher caffeine concentration could generate larger sympathetic responses and prolong its effects, but only when the caffeine level is above 200mg as suggested in literature (e.g., Brunyé et al., 2010). In the future, other physiological measures such as electrodermal activity can be a rich source of data, supporting better our conclusions (e.g., Barceló et al., 2018). Similarly, future research using double-blind tasting tasks can also combine other types of ocular measurements such spontaneous eyeblinks as they are thought to be correlated with the autonomic nervous system.

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