Effects of Adolescent Caffeine Consumption on Impulsivity Control during Adulthood

By

Maria Jose Navarro

Department of Psychology and Neuroscience, University of Colorado at Boulder

April 6, 2018

Thesis Advisor

Dr. Ryan K. Bachtell, Department of Psychology and Neuroscience

Defense Committee:

Dr. Ryan K. Bachtell, Department of Psychology and Neuroscience

Dr. Heidi Day, Department of Psychology and Neuroscience

Dr. Garrett Bredeson, Department of Philosophy
Abstract

Many people consume a variety of beverages like coffee, tea and sodas that contain caffeine as one of their principal ingredients. In recent years, adolescent caffeine consumption has increased significantly, reason why it is important to recognize and understand its effects in behaviors displayed later in adulthood. Little is known about the effects of adolescent caffeine consumption on impulsivity modulatory processes, which can play a crucial role in the development of several mental disorders. In this experiment, a rat model was used to explore the effects of adolescent caffeine consumption in a behavioral test of impulsive action during adulthood. Exposure to caffeine was done during a 28-day period during adolescence, which was followed by the application of an impulsivity behavioral test, the Differential Reinforcement at Lower levels (DRL) schedule. Our results show that rats that consume caffeine during adolescent performed better in the DRL task, exhibiting more regulation of impulsive behavior. The most outstanding differences between the caffeine and the water consuming groups were found in the efficiency index and the higher percentage of correct trials exhibited by the caffeine-consuming group. Findings reveal an early enhancement of performance in the DRL task for the caffeine consuming animals. These findings add to the existing literature on caffeine’s effects on cognitive enhancement and control of inhibition regulation.

Keywords: caffeine, adolescence, impulsivity, differential reinforcement at lower rates, delayed reinforcement, dopamine, adenosine, mesolimbic, mesocortical, control inhibition
Introduction

Caffeine is one of the most widely used substances in the United States. In its available forms, ranging from pure coffee to soft drinks, caffeine is consumed on a daily basis by a vast majority of people. Total caffeine intake in the United States is largely found in coffee, carbonated soft drinks, tea, energy drinks, energy shots, and flavored water. Although there has been a slight decline in average caffeine intake (Somogyi, 2010), a significant increase in the amount of consumers has been observed, ranging from children down to two years old to 54 years old consumers (Mitchell et al., 2014). Studies have shown that caffeine containing drinks are consumed mostly by young adults, teenagers and college students (Heckman et al., 2010). In the past decade, caffeine consumption among children and adolescents has significantly increased (Faray et al., 2005). Within this framework, this thesis assesses the effects of adolescent caffeine consumption on subsequent impulsivity-related behavior in adults.

The adolescent brain is very sensitive to changes and disruption. During early adolescent developmental stages, the limbic system which is in charge of emotional control and affective processes, matures considerably (Galdwin et al., 2011). Nevertheless, it is only until later periods of adolescence that cortical systems develop to support in advanced cognitive and regulatory skills (Reyna and Farley, 2006). The discrepancy between the maturity time of these systems, can account for the onset of developmental disorders (Ernst et al., 2006). This imbalance of systems can also contribute to vulnerability in adolescents for drugs of abuse, as the affective response is very heightened and dominant, while censoring and modulation of regulatory skills are less pronounced. Not a lot of studies have shown the possible long lasting effects of caffeine
consumption during adolescence on subsequent adult behaviors. Potential changes induced by caffeine or other stimulant use during adolescence might be revealed later in adulthood enhancing the possibility of developing mental disorders.

Caffeine belongs to the methylxanthines family and exhibits neuropharmacological properties that can be described through different modes of action. These molecular and cellular effects of caffeine result in direct behavioral and cognitive responses and may produce lasting effects on the functioning of the brain. One of the pharmacological mechanisms of caffeine is its ability to generate changes in intracellular mobilization of calcium (Bianchi, 1961). It has been theorized that large concentrations of caffeine can inhibit calcium reuptake by the endoplasmatic reticulum. However, evidence suggests that this occurs with concentrations of 200µM caffeine (Bianchi, 1968), which are not found after drinking between 1 and 3 cups of coffee. Caffeine has also been implicated with inhibition of the enzymatic breakdown of cyclic adenosine monophosphate (cAMP) (Beavo et al., 1970), an important molecule for intracellular signaling. cAMP accumulation is related to the potentiation of catecholamines (Butcher et al., 1962), which constitute an important group of organic molecules with different neurobiological functions. Nevertheless, it has been suggested that the dose needed to induce significant changes in cAMP metabolism is also quite high and near toxic concentrations that are never seen in situ experiments (Cardinal, 1980).

An important interaction of caffeine takes place at presynaptic and postsynaptic adenosine receptors that modulate both glutamate and dopamine signaling in the cortical and striatal brain areas (Ferre, 2010). In order to visualize caffeine’s effects, it is necessary to describe the actions of endogenous adenosine. Adenosine is an organic molecule with neuromodulator
properties, meaning that it plays a role in suppressing arousal and promoting sleep. In the striatum and cortical areas, adenosine acts on presynaptic A1 adenosine receptors to decrease dopamine and glutamate transmission (Orru et al., 2011). Adenosine can also dampen dopamine signaling through stimulation of either A1 and A2 adenosine receptors located on neuronal dendrites. The competitive antagonist properties of methylxanthines, like caffeine, allow them to inhibit the depressant effects produced by endogenous adenosine. In other words, caffeine has the ability to reverse adenosine-induced inhibition of glutamate and dopamine release through antagonism of presynaptic A1 receptors (Cauli and Morelli, 2005). The consequent increase in glutamate and monoamine neurotransmission could be directly related to changes in behaviors associated with the mesocortical and mesolimbic pathways (Stoner et al., 1988).

Caffeine’s pharmacological effects resemble those of stimulant drugs like amphetamine and cocaine (Atkinson and Enslen, 1976). The modulation of an increased dopaminergic signaling process, generated by stimulants, can be resembled by the actions of caffeine through its effects on adenosine receptors. These similitudes are rooted in both direct and indirect regulation of dopamine transmission, which may be involved with impulsive value and reinforcer value (Cauli and Morelli, 2005). These important modifications set the framework for important pharmacological discoveries to be made, describing the underlying dynamics of caffeine and its effects on many behavioral, cognitive and molecular processes.

In the same framework of receptor regulation and action, caffeine is known to induce neurobiological changes with chronic administration, especially when administered during specific developmental periods. For example, chronic caffeine has been implicated in alterations of cerebral adenosine receptors density. Some studies have measured an increase in adenosine
receptors in the offspring of mice that consumed caffeine during pregnancy, an effect that lasts until adulthood (Marangos et al., 1984). In comparison, juvenile rats that experienced caffeine intake during 4 and 27 days after birth, display an increase in adenosine receptors for the first two weeks after consumption stopped. (Hunter et al., 1990). Finally, caffeine has been demonstrated to generate changes in cerebral blood flow and metabolism, specifically an increase in the rate of local glucose use in dopaminergic and serotonergic areas (Grome and Stefanovich, 1985). These findings suggest that chronic caffeine during development may produce alterations in the brain that would impact the functioning of the striatal and cortical dopamine systems.

In this scenario, it is useful to study the relationship between caffeine consumption and alterations in dopaminergic system in terms of the behavioral and cognitive phenomena. Mental performance, in terms of memory and learning abilities, can be improved by caffeine consumption. Nevertheless, there is contradictory literature that recorded poor performance in cognitive tasks following caffeine consumption (Castellano, 1976). Evidence has pointed to potential capacities of caffeine to improve performance in animal studies when the subject is familiar to the environment, opposite to what happens in unfamiliar territories (Dodd et al., 1991). Nevertheless, it is important to consider the difficulty presented when studying learning behaviors, as there are many factors influencing one single and complete response (attentiveness, vigilance, performance).

Altering these molecular and biological events through the use of caffeine, specifically on systems responsible for key components of behavioral phenomena exhibited in many neurological disorders, determines a more specific experimental framework to study the
consequences of such alterations. In the context of this experiment, the behavioral and cognitive variations were measured in terms of impulsivity. Impulsivity is considered a collection of behavioral phenomena with both neuroanatomical and neuropharmacological roots, where action is done without any forethought. Impulsivity is considered a behavioral feature that is believed to contribute to several neurological disorders like drug abuse and eating disorders (Barbelivien et. al, 2008). More specifically, impulsivity is characterized by the inability to postpone an action that will lead to a reward, described as delay aversion. In that sense, impulsive actions are considered those that reflect an intolerance to wait for reward/gratification, where preference is for immediate and smaller rewards, than for delayed and bigger recompenses (Ainslie, 1975) The neuroanatomical and neurophysiological circuitry underlying impulsivity has been described in terms of alterations to the pathway between the prefrontal cortex (PFC) and the nucleus accumbens (NAc) (Cardinal, 2001), which is highly dominated by dopaminergic projections. This circuit is known to mediate appropriate behaviors and proper adaptability to situations. Malfunctioning in this system has been related to the inability to generate response inhibition (Cardinal, 2001). The primary goal of our work is to identify how adolescent caffeine consumption influences impulsivity during adulthood.

There is not a lot of scientific literature exploring adolescent caffeine consumptions and its effects later in life. The neuropharmacological principles underlying the long-lasting effects of caffeine use during adolescence that are later reflected in adulthood, are poorly understood. Based on the significant increase of adolescence consumption of easily available caffeineinated products, it is imperative to understand the potential consequences such consumption will have in the consolidation of erroneously wired neuronal circuits. In the context of this experiment, to
measure impulsivity, a specific schedule called Differential Reinforcement at Lower Rates (DRL) was used. This protocol requires animals to wait a minimum interval of time between responses (lever presses) to earn a reinforcement. The DRL schedule teases out specific measures enabling the assessment of crucial aspects of impulsivity. In one hand, the lack of inhibitory control can be displayed in the struggle the animal experiences with the increasing delay in reinforcement (Kirshenbaum et al., 2008). On the other hand, the DRL procedure can reflect heightened reward sensitivity (Dawe and Loxton, 2004). In this particular experiment, the effects of caffeine consumption during adolescence were tested with the DRL task. Two different groups of Sprague Dawley rats that were exposed to different substances during their adolescence, water or a dose of 0.3g/L of Caffeine. The DRL task was implemented during adulthood following adolescent caffeine consumption to identify any lasting effects of such consumption on impulsivity. Therefore, it was expected that rats drinking a set dose of caffeine their adolescence, would have more difficulty withholding their response in the DRL schedule, than the animals that had been drinking only water. The potential rise in neurotransmitter levels induced by caffeine consumption, could be directly involved with noticeable changes in behavior that exhibit high levels of impulsive action. This could constitute possible indicators that caffeine consumption during the developmental period of rats, could be interacting with neuronal systems that regulate pathways of reward and influence the presence of impulsivity on behavior.
Methods

Animals

Male Sprague-Dawley rats (Envigo) arrived in the laboratory on postnatal day 21 and were given a period of seven days to acclimate before caffeine administration began. Rats were double-housed and kept on a 12 – hour light/dark cycle. During the acclimation period rats were given food and water ad libitum. During the DRL experimental period, food was restricted to 4 pellets on every weekday and 6 pellets for the weekend. All behavioral tests occurred during the light period 5 days a week. All experiments and procedures were completed in accordance with parameters established by the Institutional Animal Care and Use Committee at the University of Colorado Boulder.

Caffeine administration and consumption

Caffeine consumption took place after the seven-day period of acclimation. It started on postnatal day (PND) 30 and finalized on PND 57 (Figure 1). Every animal cage contained a Hydropac (water pouch) of approximately 400 grams. Subjects were divided into two groups, a caffeine-consuming group and a control water-consuming group. A stock solution of 13 g/L of caffeine (Sigma-Aldrich, St. Louis MO) was made every week using filtered tap water. A total of 12 Hydropacs were weighed twice a week and replaced with fresh solution every Friday. Caffeine-containing hydropacs were normalized to 380 grams of water and 10 mL of the 13 g/L caffeine solution were injected into 6 Hydropacs. This resulted in a final concentration of 0.3 grams/L of caffeine. During the caffeine administration period, Hydropacs were checked twice a week for
leaks and fluid consumption was supervised every other day for the entire 28-day period. During caffeine consumption food was given *ad libitum* and body weights were recorded on a weekly basis.

**Figure 1. Caffeine Consumption Timelines.** Caffeine consumption occurred from postnatal day (PND) 30 to 57, a period encompassing adolescence in rats. Following a 1-wk washout period, rats were tested on a differential reinforcement of low rates (DRL) schedule in 15 sessions.

*Differential Reinforcement at Lower Rates (DRL) procedure*

After seven days of the last day of caffeine consumption, the DRL procedure was performed in operant conditioning chambers (Med Associates, St Albans, VT) that contained one response lever, a house light, a stimulus cue light and a pellet delivery magazine. The DRL model consisted of one hour sessions during which animals could perform a lever response to acquire a banana-flavored sucrose pellet on a Fixed Ratio 2 (FR2) schedule of reinforcement. In order to successfully earn a sucrose pellet, rats were required to withhold the 2<sup>nd</sup> lever response for a specific interresponse time (IRT) (Figure 2). Five successful trials, each of which required the rat to successfully perform the FR2 schedule and acquire the sucrose pellet, constituted one block. Each block corresponded with a specific IRT with the 1<sup>st</sup> block starting with a 0.5 sec IRT. Each subsequent block increased the IRT by 0.5 sec (0.5, 1.0, 1.5, 2.0, 2.5, etc.). The beginning of each trial was signaled by a house light, which was terminated upon the 1<sup>st</sup> lever response and yellow
stimulus light was illuminated during the IRT. If the 2nd response occurred after the specified IRT, a sucrose pellet was delivered, the stimulus light terminated, and the configuration of the chamber reset. If the 2nd response occurred during the IRT, the stimulus light would turn off and the trial would restart. Each session ended after either an hour had elapsed or 20 blocks were completed. Every daily session started from block #1 and with an IRT of 0.5 seconds.

![Diagram](image)

**Figure 2. Differential Reinforcement at Lower Rates (DRL) procedure.** a) One successful trial consisted in two lever responses separated by a determined interresponse time (IRT). The animal was expected to submit an initial response and withhold their second response for the required IRT in order to acquire a reward (R). Successful execution of 5 trials, constituted one block. The first block started with and IRT of 0.5 sec and increased by 0.5 sec per block completed. b) If the second response was done before the corresponding IRT, no reward was delivered, and the configuration of the box would reset, leading to a new trial.

**Data Collection and Analysis**

Each session resulted in several dependent variables that were analyzed including the number of blocks completed, total premature responses, percent of correct trials, latency to
initial response and latency to reward retrieval. An efficiency index was calculated as the ratio of correct responses to total responses. Each dependent variable was analyzed separately across sessions by comparing the caffeine consuming group with the control group that only had access to water. We also binned the sessions into early, middle and late phases by averaging each dependent variable across 5 consecutive sessions. This provided Two-way ANOVA tests that were used to evaluate the effects of adolescence caffeine consumption in the above-mentioned variables with consumption group (between) and sessions (within) as the parameters to determine differences in behavior that can exemplify impulsive patterns, induced by caffeine consumption.

Results

Caffeine Consumption

During the caffeine administration and consumption period, we closely monitored each animal’s body weight and fluid intake. There was an observed increment in the amount of fluid consumed that was comparable between experimental and control group. This was supported by significant main effect of Days (Figure 3a, $F_{13,130} = 22.47$, $p < 0.0001$) and no significant effect of Group or the interaction between these variables. As expected, body weight steadily increased throughout the adolescent consuming period (Figure 3b, $F_{154} = 1728$, $p < 0.0001$), with no statistical difference in trend between caffeine consuming group and water-only consuming group. Using body weight, fluid consumed and the concentration of the caffeine containing
water, we calculated the dose of caffeine consumed. A significant decline in the dose of caffeine consumed (27.11 ± 1.35mg/kg) was observed (F_{13,65} = 10.75, p < 0.001; Figure 3c).

**Effects of Adolescent Caffeine Consumption on the Differential Reinforcement at Lower Rates Task**

This experiment tested the effects of caffeine consumption during adolescence on the DRL task, which evaluates the capacity of an animal to adapt to the increasing delay in reinforcement and is considered a measure of impulsive action. To evaluate the overall effects of adolescent caffeine consumption, we analyzed the number of blocks completed during each session. This provided a basic measure of adaptive performance on the DRL task, directed by the ability of the animal to adapt to the increment of the IRT associated with each block. The progressive increase of blocks completed per individual session reflected the adjustment of lever-response that animals where developing in each required IRT. Adolescent caffeine consumption did not have an overall significant effect on the number of blocks completed. Surprisingly,
performance on this measure remained constant between each session with no significant
effects of session or interaction between consumption and session (Figure 4a.). In the same
manner, no overall significant effects of caffeine consumption were observed in the averaged
numbers of blocks completed when the blocks completed were binned across five consecutive
sessions. (Figure 4b.). We also recorded the total number of correct lever responses between
groups across the 15 sessions. No significant effects of adolescent consumption were observed.
In the same manner, no effects of interaction between consumption and session performance
was observed (Figure 4c.). Similarly to the number of blocks completed, the caffeine consuming
groups shows a slight initial recording of correct responses, but no effect of treatment or session
was observed in the average number of correct responses between early, middle and final
sessions. (Figure 4d.)
Figure 4. Generalized measures of performance on the DRL task. 

(a) Number of Blocks Completed during all 15 sessions of the DRL task. Each completed block required five successful trials, during which the animal had to withhold the lever-pressing response for each block’s corresponding interresponse time (IRT). The IRT progressively increased by 0.5 sec throughout each individual session. No significant effects of treatment ($F_{1,266} = 0.3159, p = 0.3849$), session ($F_{14,266} = 0.8157, p = 0.6519$) or interaction ($F_{14,266} = 0.3159, p = 0.9918$) were observed.

(b) Averaged number of blocks completed in the DRL task were averaged across the early (1-5), middle (6-10) and late (11-15) sessions. Each column represents the average number of completed blocks in five consecutive sessions per group. No significant effect of treatment ($F_{1,40} = 1.582, p = 0.2229$), session ($F_{2,40} = 0.9301, p = 0.4029$) or interaction ($F_{2,40} = 0.1939, p = 0.8245$) were observed.

(c) Total Correct Lever Responses during the DRL task. These were responses that occurred after the specified IRT, leading to moving up in number of blocks. No significant effect of treatment ($F_{14,266} = 0.7910, p = 0.3849$), session ($F_{14,266} = 0.8157, p = 0.6519$), or interaction ($F_{14,266} = 0.3159, p = 0.9918$) between caffeine consumption and session performance were recorded.

(d) Average of total correct responses in early, middle and final sessions. No significant effect of treatment ($F_{1,40} = 2.029, p = 0.1697$), session ($F_{2,40} = 0.2484, p = 0.7812$) or interaction ($F_{2,40} = 0.2650, p = 0.7685$) were observed.
To further analyze the effects of adolescent caffeine consumption on impulsive patterns of behavior, we recorded the total number of premature responses. These are responses made during the IRT that resulted in a failed trial. Although there was no overall significant effect of adolescent caffeine consumption on premature responding, there was a significant main effect of session (Figure 5a., $F_{14,266} = 3.096$, $p < 0.001$) and a significant interaction between consumption and session (Figure 5a., $F_{14,266} = 3.086$, $p < 0.001$). This suggests the possibility of a treatment effect on the early sessions of the experiment, where caffeine consumption appears to be associated with reduced premature responding, effect that normalizes between groups after the early sessions. This notion is reinforced by the significant interaction between adolescent caffeine consumption and premature responses where adolescent caffeine consumption contributed to lower numbers of premature responses in the first five sessions of the experiment (Figure 5b., $F_{2,38} = 6.805$, $p = 0.0030$).

**Figure 5. Measures of impulsive action during the DRL task.** a) Total Premature Responses during the DRL task across all 15 sessions. Premature responses were responses complete before the required IRT of the corresponding block and resulting in a failed trial. No overall effect of treatment was observed ($F_{1,266} = 0.575$, $p = 0.4573$), although there was a session and interaction effect of adolescent caffeine consumption. b) Averaged Total Premature Responses during the DRL task were averaged across the early (1-5), middle (6-10) and late (11-15) sessions. No significant effects of treatment ($F_{1,38} = 0.5757$, $p = 0.4573$) or session ($F_{2,38} = 0.0389$, $p = 0.9618$) were observed.
Furthermore, performance in the DRL task was evaluated by quantifying the fraction of correct trials to total number of trials per individual session. Adolescent caffeine consumption had an overall significant effect on the percentage of correct trials (Figure 6a, $F_{1,252} = 7.133, p < 0.0156$), and the percentage of correct trials also varied significantly over sessions (Figure 6a, $F_{14,252} = 4.709, p < 0.001$). Similarly, a significant interaction between caffeine consumption and session performance (Figure 6a, $F_{14,252} = 3.313, p < 0.001$) suggested that in the early sessions, adolescent caffeine consumption seems to be associated with higher percentage of correct trials. This was observed in the significant effect of treatment (Figure 6b, $F_{1,40} = 10.07, p = 0.0048$) in the early sessions, compared to middle and late sessions. There was also an observed effect of interaction between the averaged percent of correct trials in the first five sessions (Figure 6b, $F_{2,40} = 4.885, p = 0.0126$). Finally, performance was also measured using an efficiency index, which was calculated as the percentage of correct responses out of all responses. No significant overall effect of adolescent caffeine consumption was observed, although there was a significant main effect of session (Figure 6c, $F_{14,252} = 4.302, p < 0.0001$). Similarly, there was an observed effect of interaction between caffeine intake and session performance across groups (Figure 6c, $F_{14,252} = 3.235, p < 0.0001$). Although there was no significant effect of caffeine consumption in the averaged efficiency indexes across early, middle and late sessions, a significant interaction between caffeine consumption and session performance was recorded in the first five sessions (Figure 6d, $F_{2,38} = 5.676, p < 0.0070$).
Figure 6. Impulsivity performance measures on the DRL task. (a) Percent of Correct Trials in the DRL task was analyzed across all 15 sessions. These were calculated as the fraction of correct trials, to total number of trials in each individual session. Caffeine consumption was associated with a higher percent of correct trials, reflected in the significant effect of treatment ($F_{1,252} = 7.133$, $p < 0.0156$). An effect of session was also recorded, ($F_{14,252} = 4.709$, $p < 0.001$), similarly to a significant interaction ($F_{14,252} = 3.313$, $p < 0.001$) between caffeine consumption and sessions performance. (b) Percentage of Correct Trials during the DRL task of early (1-5), middle (6-10) and late (11-15) sessions. An overall effect of caffeine consumption was identified ($F_{1,40} = 10.07$, $p = 0.0048$), as well as an effect of interaction between adolescent consumption and session development ($F_{2,40} = 4.885$, $p = 0.0126$), meaning that caffeine consuming group exhibited higher percentage of correct trials in the first five sessions. (c) Efficiency Index in the DRL task across the 15 sessions. The Efficiency Index was calculated as the total number of correct responses to total number of responses. Caffeine consumption was associated with a higher level of efficiency. Although there was no significant effect of treatment, an effect of sessions was identified ($F_{14,252} = 4.302$, $p < 0.0001$). A significant interaction between consumption and efficient performance was also revealed ($F_{14,252} = 3.235$, $p < 0.0001$). (d) Efficiency index during the DRL task of early (1-5), middle (6-10) and late (11-15) sessions. No overall effect of caffeine consumption was measured in any of the session groups ($F_{1,38} = 1.621$, $p = 0.2183$), but a significant interaction between increased performance and caffeine consumption was measured ($F_{2,38} = 5.676$, $p < 0.0070$).
Discussion

This study determined the effects of adolescent caffeine consumption on impulsive action during adulthood using the DRL task. The DRL model provides various dependent variables that can reveal various aspects about impulsive action. Reasonably, the number of blocks completed resembled the number of total correct responses, as each block completion was determined by the completion of five successful trials, each of them encoding a correct response. Changes in premature responding, percent of correct trials and efficiency index exposed a plausible relation between caffeine consumption during adolescence, and enhanced performance in the DRL schedule. The general trends in the results point towards the possibility that caffeine consumption during adolescence enhances inhibition control, cognitive performance and impulsive action regulation. In the larger scheme of scientific literature present on this topic, the effects of caffeine on behavioral models of impulsivity and cognitive performance can be potentially sorted in two main different categories: the conception that caffeine can act as a cognitive stimulator and reduce impulsivity cues, as well as having no significant effect on the regulation of impulsivity.

Impulsive choice has been implicated in several clinically relevant mental conditions, including drug abuse, personality disorders, mania and Attention Deficit and Hyperactivity disorder (ADHD) (American Psychiatric Association, 2000). Different experimental models, both human and nonhuman, have validated the experimental significance of evaluating impulsivity with delay or temporal discounting models (Mazur, 1987). This behavioral test devalues the reinforcer by introducing a delay in reinforcement presentation. In contrast with the DRL task, delay discounting usually presents two reinforcers: one small reward delivered immediately upon
correct responding, or large reward that is delayed following correct responding. The self-
controlled choice is considered the one where there is a preference for the delayed reward, while
impulsive choice is the one that prefers instant reinforcement. Evidence points to important
correlations between caffeine consumption and reduction of choice of the small reward,
compared to the larger delayed reward. An experiment where caffeine consumption was done
during the delayed reinforcement tasks exhibited a decrease of impulsive choice in the caffeine
consuming group (Diller et al., 2008). Our laboratory has performed similar experiments where
caffeine consumption occurs during adolescence, and similar conclusions have been reached:
caffeine enhances performance on the delayed discounting task (unpublished observations, TAL
& RKB). Our findings support these studies using a model of impulsive action. In the context of
the DRL model, there is no choice between small immediate reinforcement or large delayed
reinforcements. Instead, impulsive action is measured by the animal’s adaptability to the
increasing delay between two responses. For instance, during early sessions, the caffeine
consuming group adapted easily to the delay in reinforcement, seen in the higher efficiency
index. This means that the caffeine group exhibited a higher preference for withholding their
response in order to get a reward, leading to a higher fraction of correct responses to total
responses, compared to the water-drinking group.

As the DRL model reinforces behavior in lower rates and the imposition of the IRT
becomes more demanding for the animal, the difficulty of adaptation increases as the desire to
produce the response is more influential. In that sense, the cognitive process underlying the
selection of the delayed reinforcer is consolidated via control inhibition. In the present study,
within-block performance was found to be significantly better in the caffeine consuming group
compared to control. Inappropriate behavior (i.e., Premature responses or responses done before IRT was achieved) was less prevalent in the caffeine group, not only in comparison with the water group, but within session as well. The higher percent of correct trials exhibited by the experimental group was another sign of enhanced performance displayed by the caffeine consuming group. Interestingly, enhancement was predominantly recorded in the early stages of DRL testing, which could point to the necessity of caffeine consumption during adulthood in order to generate more long-lasting changes in impulsivity modulation process. This poses important questions directed to the durability of the caffeine enhancement effects, as well as their directionality. In other words, it is unclear whether the caffeine consuming group started the DRL task with an enhanced performance due to improved learning, more than to inhibition control. Caffeine could be directly interacting with learning systems more predominantly than with regulatory systems, reason why there is no long-lasting enhancement of performance induced by caffeine consumption. On the other hand, it could also be possible that longer withdrawal periods are responsible for the weakening of caffeine’s enhancement properties. Future research can study the effects of acute caffeine consumption during both adolescence and adulthood, by subjecting animals to behavioral tests while consumption is still continued. Equally, consumption can be done either during childhood or late stages of adolescence. Altering the time of consumption could be the key to identify the moment where important neuronal changes are consolidated, and the moment of development that can be the most vulnerable to disruption.

Subsequently, the observations collected from the present experiment further support findings that display enhancing properties of caffeine in the regulation of impulsive behaviors. Particularly, some studies have explored the effects of caffeine on reducing the neuronal
dysfunction distinctive of ADHD using delayed reinforcement models. ADHD is a mental disorder characterized by attention deficits, impulsivity and hyperactivity. The most recognized experimental design for ADHD testing in animals is the hyperactive rat (SHR) (Sagvolden et al., 2009). It has been shown that caffeine can resemble some properties of methylphenidate, the first drug choice for ADHD, by stabilizing the under-functioning seen in motor and executive brain regions, specially frontocorticostrial connections (Jonkman et al., 2007). For instance, caffeine improved spatial learning deficits seen in SHR rats (Prediger et al., 2005), which can be related to poor dopamine signaling between the frontal cortex and the striatum (Arnsten, 2006). Caffeine improves dopamine signaling by blocking adenosine receptors that typically counteract dopamine receptor signaling in striatal and frontocortical neurons (Schiffman et al., 2007).

Caffeine’s action on adenosine neuromodulation of dopamine and glutamate signaling, especially between the striatum and the frontal cortex, might be key in understanding the possible effects that adolescent caffeine consumption can have in the regulation of impulsivity present later in adulthood. Pandolfo et al., (2013) reports that caffeine consuming adolescent male SHR rats exhibited a reduction in the dopamine transporter (DA), which is in charge of dopamine’s uptake in synaptic areas. Equally, it was shown that caffeine dampens the increased density of adenosine A2A receptors, especially in dopaminergic terminal areas. Caffeine’s modifications of adenosine and dopamine signaling in the adolescent brain could potentially be responsible for important adjustments in impulsivity regulation. This could explain the predominantly early enhancement that the caffeine consuming group displayed in the present study. Both efficiency index and percentage of correct trials was significantly higher for the experimental group in early sessions. It is possible that in order to generate long-lasting effects
in impulsivity regulation, caffeine consumption has to be either chronic or deep into adulthood. Nevertheless, it is important to recognize the opposite results some studies reveal. Evidence has pointed that caffeine’s reversal effects on neuronal dysfunction present in hyperkinetic disorder were not significantly different compared from those of the placebo drug, opposite of what happens with stimulants like d-amphetamine (Huestis et al., 1975). Hyperkinetic disorder is a neurodevelopmental disorder that emerges in early childhood, characterized by hyperactivity and impulsivity.

It is important to note key differences between previous studies and the present study, especially with respect to timing, route of administration, and dosage; variables that could influence subsequent performance in the DRL schedule. One important difference is the durations of caffeine consumption. The current study administered caffeine orally during a 28-day adolescent period and behaviors were tested in the absence of caffeine during adulthood. Many other studies administered caffeine during the behavioral and cognitive tests to reveal the direct effects of caffeine on behavioral performance. Future studies may better capture the long-term effects of caffeine using a mixture of these experimental designs where either continued or acute administration of caffeine may reveal the long-lasting effects on impulsivity control. Similarly, dosage is another factor that could account for different reported effects of caffeine. The results of the present study report an average of caffeine consumption of 27.11 ± 1.35mg/kg, similar to what Kirch et al. (1990) use in their studies. They reported an increase in dopamine and serotonin levels in the striatum after repeated administration of relatively high doses of caffeine (25mg/kg and 50mg/kg), while no significant effects in monoamine levels were observed when low doses (10mg/kg) were given. With that being said, duration and dosage can be correlated
variables responsible for significant changes both in molecular processes and cognitive performance in behavioral tests.

Important conclusions can be drawn from the present experiment and the literature that accompanies it. Future research should investigate the effects of different models of caffeine administration, in different stages of adolescence as well as into early stages of adulthood. Different doses might also be needed in order to identify the variety of potential effects of caffeine, which could influence molecular regulatory processes in the frontal cortex and striatum. Underlying these processes represents an important advancement in the pharmaceutical significance of caffeine, considering its wide and constant use, as well as an advancement in the understanding of impulsivity and the neuronal process it underling it.
References


American Psychiatric Association Diagnostic and statistical manual of mental disorders,


Atkinson, J. and Enslen, M., Self-administration the rat, Arzneimittelforsch., 26 (1976) 2059-2061


Bizot, J.C., Chenault, N., Houze, B., Herpin, A., David, S., Pothion, S., Trovero, F.


Cardinali, D.P., Methylxanthines: possible mechanisms of action in brain, Tr. Pharmacol. Sci.,


Evenden J.L., Ryan C.N. The pharmacology of impulsive behaviour in rats: the effects of drugs on response choice with varying delays of reinforcement Psychopharmacology, 128 (1996), 161-170


Ferré S.J., Role of the central ascending neurotransmitter systems in the psychostimulant effects


Gadaire, M., Dana, Marshall G., Brissett E., Differential reinforcement of low rate responding in social skills training, Learning and Motivation, 60 (2017) 34-40


Jentsch J. D., Taylor J. R., Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. Psychopharmacology
146 (1999) 373–390


Lin, Y. and Phillis, J.W., Chronic caffeine exposure reduces the excitant action of acetylcholine on cerebral cortical neurons, Brain Res., 524 (1990) 316-318


Manalo, Rafael V. M., and Paul M. B. Medina, Caffeine Protects Dopaminergic Neurons From Dopamine-Induced Neurodegeneration via Synergistic Adenosine-Dopamine D2-Like Receptor Interactions in Transgenic Caenorhabditis Elegans, Frontiers in Neuroscience 12 (2018) 137


Marangos, P.J., Boulenger, J.P. and Pate1 J., Effects of chronic caffeine on brain adenosine


Rusted J. Caffeine and cognitive performance: Effects on mood or mental processing. In: Gupta B.S., Gupta U., editors. Caffeine and behavior: Current views and research


