

## Review

# Effects of caffeine consumption on Attention Deficit Hyperactivity Disorder (ADHD) treatment: a systematic review of animal studies.

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**Abstract:** Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by a persistent pattern of inattention and/or hyperactivity-impulsivity. Neurobiologically, ADHD impairments arise from abnormalities in different circuits involving the prefrontal cortex. In face of high rates of diagnosis, alternative/complementary pharmacological therapeutic approaches for ADHD are needed. Although the number of publications that study the potential effects of caffeine consumption on ADHD treatment have been accumulating over the last years, and caffeine has recently been used in ADHD research in the context of animal models, an updated evidence-based systematic review on the effects of caffeine on ADHD-like symptoms in animal studies is missing. To provide insight and value at the preclinical level, a systematic review based on PRISMA guidelines was performed for all publications available up to September 1, 2021. Caffeine treatment increases attention, improves learning, memory and olfactory discrimination, without altering blood pressure and body weight. These results are supported at the neuronal level. Nonetheless, the implication of caffeine in modulating ADHD-like symptoms of hyperactivity and impulsivity is contradictory, raising discrepancies that require further clarification. Our results strengthen the hypothesis that caffeine cognitive effects found in animal models could be translated to human ADHD, particularly during adolescence.

**Keywords:** caffeine; attention deficit hyperactivity disorder; impulsivity; ADHD; animal models

## 1. Introduction

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by a persistent (no less than 6 months) pattern of inattention and/or hyperactivity-impulsivity, inconsistent with developmental level, that negatively impacts in at least two settings (academic, occupational or social) [1]. On the one hand, inattention manifests behaviorally in ADHD as wandering off task, lacking persistence, having difficulty sustaining focus, and being disorganized, and is not due to defiance or lack of comprehension. On the other hand, while hyperactivity refers to excessive motor activity when it is not appropriate, or excessive fidgeting, tapping or talkativeness, impulsivity relates to hasty actions that occur at the moment without forethought, have high potential for harm to the individual and may reflect a desire for immediate rewards or an inability to delay gratification [1]. Depending on the symptoms presented, three different types of ADHD can be diagnosed: predominantly inattentive presentation, predominantly hyperactive-

impulsive presentation, or combined presentation. Although ADHD onset occurs during childhood -with evidence of significant inattention and/or hyperactivity-impulsivity symptoms before age 12- it often persists into adulthood. Population surveys suggest that ADHD occurs in most cultures in about 5% of children and about 2.5% of adults [1]. In face of these rates of diagnosis, alternative/complementary pharmacological therapeutic approaches for ADHD are needed.

At the neurobiological level, it is hypothesized that ADHD impairments arise from abnormalities in different circuits involving the prefrontal cortex [2]. Sustained attention is hypothetically modulated by a cortico-striato-thalamocortical (CSTC) loop that involves the dorsolateral prefrontal cortex (DLPFC) projecting to the striatal complex. Selective attention is modulated by a cortico-striato-thalamo-cortical (CSTC) loop arising from the dorsal anterior cingulate cortex (dACC) and projecting to the striatal complex, then the thalamus, and back to the dACC. Impulsivity is associated with a cortico-striato-thalamocortical (CSTC) loop that involves the orbitofrontal cortex (OFC), the striatal complex, and the thalamus. Finally, motor activity, such as hyperactivity and psychomotor agitation or retardation, can be modulated by a cortico-striato-thalamo-cortical (CSTC) loop from the prefrontal motor cortex to the putamen (lateral striatum) to the thalamus and back to the prefrontal motor cortex [2]. ADHD patients generally cannot activate prefrontal cortex areas appropriately in response to cognitive tasks of attention and executive functioning. This inefficient information processing is hypothetically caused by imbalances in dopamine (DA) circuits in the prefrontal cortex [2-3]. Animal studies have provided insights into the pathological and neurochemical basis of ADHD, through different types of animal models (see Figure 1) [4]. Prominent among them, the spontaneously hypertensive rat (SHR) is considered an excellent and validated hyperactive model to study ADHD. Concerning its behavioral profile, SHR presents anomalies in DA neurotransmission [5] and, importantly, in adenosine neurotransmission [6].



FIGURE 1 | Animal models for Attention Deficit Hyperactivity Disorder

Caffeine, in this respect, is an adenosine A<sub>1</sub> and A<sub>2A</sub> receptor antagonist controlling synaptic plasticity [7]. These receptors are functionally coupled with certain postsynaptic DA receptors, such as DA 2 (D<sub>2</sub>) receptors, at which DA binds and has a stimulatory effect. When adenosine binds to its receptors, this causes reduced sensitivity of D<sub>2</sub> receptors. Antagonism of adenosine receptors by caffeine prevents adenosine from binding, enhancing dopaminergic actions [2]. Nevertheless, the effects of caffeine consumption on ADHD treatment remains largely controversial. On the one hand, there is an existing correlation between daily consumption of moderate doses of caffeine and related benefits in different psychiatric disorders linked with adenosine A<sub>2A</sub> receptor blockade controlling synaptic

plasticity [8], mainly at glutamatergic synapses [9]. Moreover, regular coffee consumption improves children’s performance in comparison to decaffeinated coffee or placebo [10]. On the other hand, some studies have reported that caffeine consumption improvement is not significantly superior to placebo [11] or methylphenidate (MPD) [12]. Moreover, hyperactivity has been strongly associated with a higher coffee consumption among adolescents [13].

The number of publications that study the potential effects of caffeine consumption on ADHD treatment have been accumulating since 1975 (see Figure 2) and, over the last few years, caffeine has been used in ADHD research in the context of animal models. Surprisingly, an updated evidence-based systematic review on the effects of caffeine on ADHD-like symptoms in animal studies is missing.

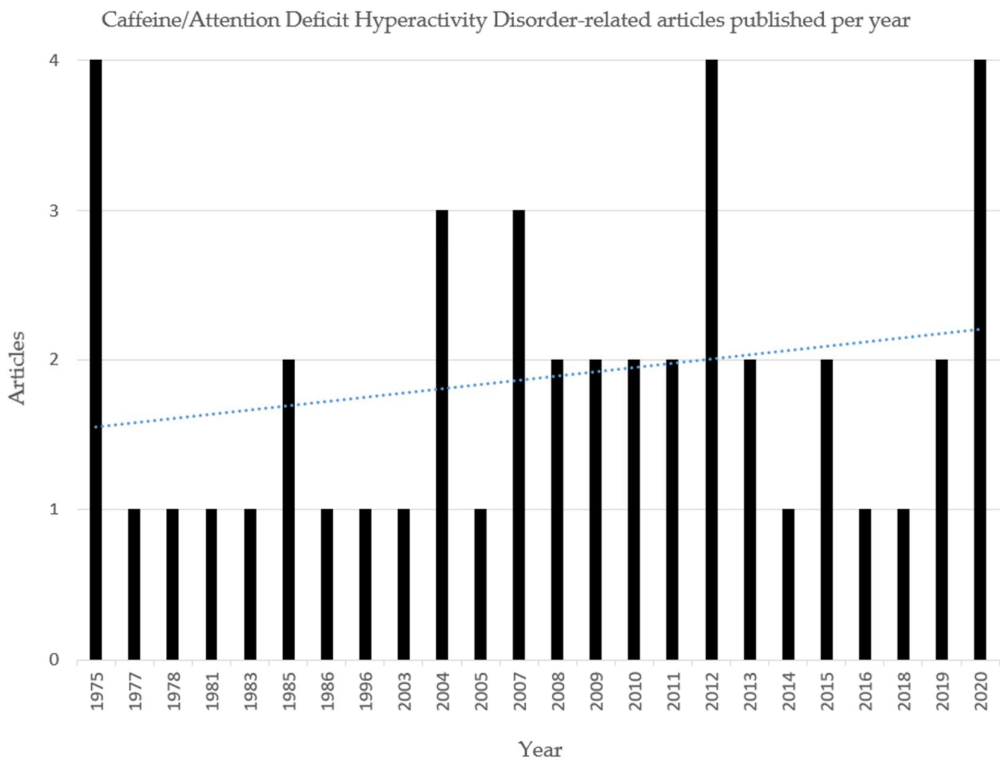


FIGURE 2 | Caffeine/Attention Deficit Hyperactivity Disorder-related articles since 1975. (Source: MEDLINE).

Therefore, to provide insight and value at the preclinical level, we sought to make a comprehensive compilation and systematically review all relevant scientific publications that make reference to the underlying effects of caffeine consumption on treating ADHD-like symptoms in animal studies.

2. Materials and Methods

We conducted a systematic review of ADHD research in the context of animal models to evaluate the association between caffeine and ADHD dependent variables including attention, locomotor activity, impulsive behavior, learning and memory.

### 2.1. Search strategy

Figure 2 depicts the search strategy. We followed the guidelines and recommendations contained in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [14], in order to reliably structure the gathered information in this systematic review. Academic articles were located using two electronic databases: MEDLINE and Web of Science. Only the results from these two databases were reported, since results from other sources (Scopus, Google Scholar) did not provide any relevant new results. No restrictions regarding publication date were applied. The literature search was conducted on September 5<sup>th</sup> 2021.

According to our proposal, the MEDLINE search strategy was based on the following key search terms: "caffeine"[Mesh] AND "Attention Deficit Disorder with Hyperactivity"[Mesh]. MeSH (Medical Subject Headings) terms were therefore used in the development of this search. The Web of Science search strategy was based on the following key search terms: ("attention" OR "hyperactivity" OR "ADHD") AND "caffeine".

### 2.2. Study selection criteria

The search was limited to preclinical and original experiments in non-human animals. The inclusion criteria were: (1) indexed studies written in English; (2) preclinical/experimental studies performed on non-human animals; (3) the mention of the relationship between caffeine treatment and ADHD-like symptoms; and (4) controlled studies with separate treated groups. The exclusion criteria were: (1) clinical/experimental/qualitative studies on humans; (2) not mentioning at all caffeine treatment and ADHD-like symptoms; (3) reviews, posters, conference abstracts, oral speeches, commentaries, theoretical papers, unpublished relevant studies, other studies relevant to the topic but not published in peer-reviewed journals; and (4) case and cross-over studies.

### 2.3. Study selection

Duplicates of all databases were removed. Titles and abstracts were independently screened by two authors (J.V.C. and O.M.T.) according to the inclusion and exclusion criteria. Articles interpreted as compatible were selected for a full-text analysis to determine whether they were or were not within the inclusion criteria. Furthermore, the references of selected studies were screened in search of additional articles that met the inclusion criteria. Whenever a divergence of opinions emerged, a third author (D.R.R.) was consulted to discuss and reach an agreement between the authors.

### 2.4. Data extraction and analyses

After selecting the studies, the data was extracted and organized into a table. The following information was collected: (1) author/s & year; (2) species: strain, sex & sample (n); (3) animal model; (4) age & lifespan development; (5) independent variables; (6) caffeine treatment; (7) behavioral tests / types of stress; (8) dependent variables; and (9) main results.

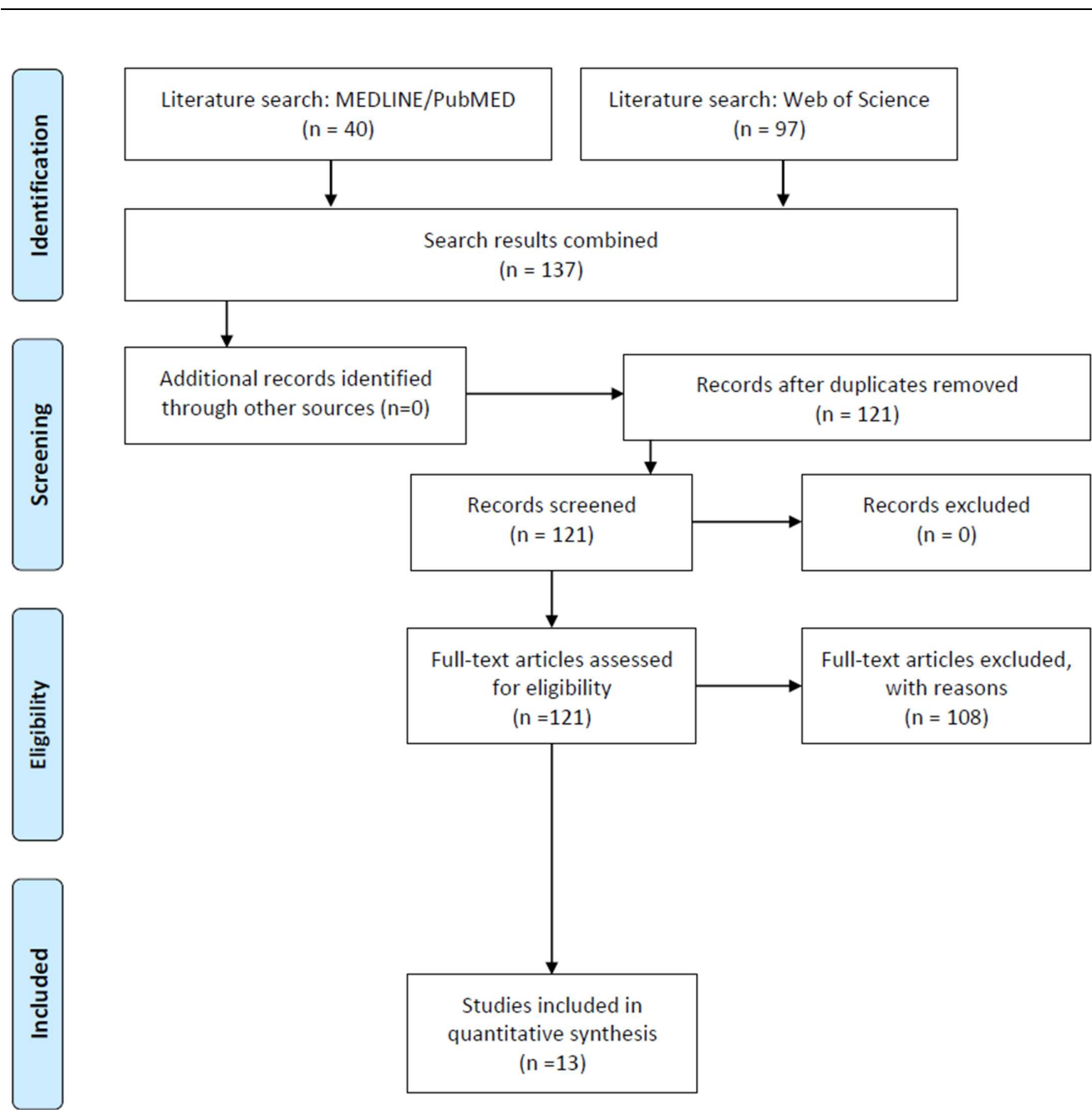


FIGURE 3 | Flow diagram of study's selection based on PRISMA guidelines [14]

### 3. Results

Due to the large number of results obtained by the search terms, strict inclusion/exclusion criteria were applied to limit the final selection of studies. Figure 2 shows the studies included in quantitative synthesis.

#### 3.1.1. Study selection

A total of 121 unique citations were initially retrieved through the combined search, of which 108 were excluded after full-text screening because they did not meet the inclusion criteria. Therefore, 13 studies (França et al., 2020 [20]; Alves et al., 2020 [27]; Leffa et al., 2019 [25]; Ruiz-Oliveira et al., 2019 [18]; Nunes et al., 2018 [21]; Szczepanik et al., 2016 [22]; Ouichi et al., 2013 [16]; Pandolfo et al., 2013 [15]; Caballero et al., 2011 [17]; Pires et al., 2010 [23]; Pires et al., 2009 [26]; Higgins et al., 2007 [19]; Prediger et al., 2005 [24]) on animal models were finally considered. Based on their methodology, studies in this review could be classified as experimental (n = 10; 76.9%), randomly assigning the subjects sample to the experimental groups, and quasi-experimental (n = 3; 23.1%), where the groups were usually constructed according to the subject's characteristics. The first studies relevant to the topic were from 2005, while the most recent studies included in this review were published in 2020. Table 1 describes each article individually.

### 3.1.2. *Species, animal model, sex, and treatment*

Most of the animal studies were conducted in rodents. Ten studies were conducted with rats and two with mice. Only one of the studies used zebrafish as an animal model. Four studies used only males, five used both males and females, and four used only females. Different caffeine treatments were studied, as well as the route of administration and the duration of treatments (Table 1). Chronic treatments were mainly done by diluting caffeine powder in the system water, whereas acute ones were mainly administered intraperitoneally (i.p.).

### 3.1.3. *Animal models of ADHD*

Overall, 9 studies used genetic animal models of ADHD: 8 studies used the SHR and 1 study used the low-density lipoprotein receptor (LDLr) mouse. Finally, 2 studies used physical trauma to provide an epigenetic animal model of ADHD: 1 study caused 6-hydroxy-dopamine (6-OHDA) lesions in rats, while 1 study used social isolation (SI) as an intensely stressful environment in mice (Table 1).

### 3.1.4. *Behavioral tests*

Five studies used the object recognition task; four studies used the Y maze test; three studies used the open field test; two studies used the water maze test; two studies used the novel object recognition test. In addition, other tests were performed, including the water finding test, the 5-choice serial reaction time task (5-CSRTT), the locomotor activity test, the discrimination task, the Olton maze behavioral assay, the attention set-shifting task, the fear-conditioning test, the tolerance to delay of reward task or the olfactory discrimination test. One study also submitted the animals to a certain type of stress, such as social isolation or aggressivity (Table 1).

### 3.2. *Study Outcomes*

The results are summarised in Table 1. Considering the amount of data provided in the reviewed articles, we decided to categorize all the information based on the caffeine effects on each relevant ADHD-like evaluated parameters, as follows:

### 3.2.1. Attention

#### *3.2.1.1. Attention and behavioral flexibility*

P. Pandolfo et al. [15] tested the impact of a chronic treatment with caffeine during adolescence on the performance of SHR and Wistar Kyoto (WKY) rats in an attention set-shifting task, a test placing emphasis on response conflict, requiring the animal to shift attention from one stimulus dimension to another. The protocol was divided into three main different phases: familiarization, response discrimination and visual cue discrimination. The response discrimination phase required that rats should always turn in a direction opposite to the initial turning bias to retrieve a food pellet, regardless of the position of the randomly selected start arm (east, west, or south) or the placement of the visual cue. During this phase, statistical analysis indicated that vehicle-treated SHR needed more trials to reach the criterion of 10 consecutive correct choices when compared with WKY rats. Importantly, caffeine treatment (2 mg/kg, i.p.) improved the discriminative learning selectively in SHR, as indicated by a reduction in the number of trials required to reach the criterion, whereas caffeine was devoid of effects in WKY rats. The visual cue discrimination phase required that rats should always turn into the arm containing the visual cue to retrieve a food pellet, regardless of the position of the randomly selected start arm (east, west, or south) or the rat's turning bias. During this phase, similar results were reached. SHR required more trials to learn the task compared with WKY rats. Again, treatment with caffeine (2 mg/kg, i.p.) reduced the number of trials required to reach the criterion. Finally, the statistical analysis revealed that vehicle-treated SHR made significantly more regressive and never-reinforced errors than vehicle-treated WKY rats. Notably, caffeine treatment (2 mg/kg, i.p.) reduced the number of these errors in SHR, while having no effect in WKY rats.

#### *3.2.1.2. Spatial attention*

H. Ouchi et al. [16] examined the effect of SI on latent learning, as an index of spatial attention, using the water-finding test, and measuring entering latency and drinking latency. The authors finally discussed the availability of SI as an epigenetic model of ADHD. They socially isolated male or female Institute of Cancer Research (ICR) mice for 1 week or more. As a result, animals exhibited spatial attention deficit in the water-finding test. Re-socialized rearing for 5 weeks after 1-week SI failed to attenuate this spatial attention deficit. Drinking latency depended on how much the animal paid attention to environmental factors, including the location of a tap water nozzle, which they were exposed to in the training trial. Therefore, a decrease in drinking latency correlated with the animal remembering the position/location of the nozzle. Caffeine (0.3–1 mg/kg, i.p.) induced changes of drinking latency in the water finding test, in this sense, significantly ameliorating SI-induced latent learning deficits in a dose-dependent manner, independently of gender or age.

Caballero et al. [17] studied the possible therapeutic use of caffeine in a different animal model of ADHD, namely the neonatal 6-OHDA lesioned rat. At postnatal day (PND) 7 rats were lesioned at the left striatum with 6-OHDA. At PND 25 spatial attention was measured with an eight arm radial maze, the Olton maze. Animals were placed in the



maze. The total number of arms the animals walked before completing 6 out of 8, or until they repeated one of them, was measured. Caffeine was then administered *ad libitum* in the drinking water. After 14 days of caffeine treatment, the authors assessed the effect of caffeine on attention deficit using the same task. Interestingly, a significant improvement in the attention deficit of the 6-OHDA lesioned rats was achieved after caffeine treatment. Since caffeine had a beneficial effect on the spatial attention of the rats, the authors finally hypothesized that caffeine might be useful to manage the attention deficit during the pre-pubertal period of ADHD.

### 3.2.1.3. Discrimination

Ruiz-Oliveira et al. [18] evaluated the effect of caffeine on zebrafish performance in a task requiring focus and attention, the discrimination task. The task took place in three phases: tank acclimation, training, and test. The authors used visual cues (target and distractors) during the training and test trials. Distractors, objects resembling the target, were used to confuse the fish and impair conditioning. Fish were exposed to three caffeine concentrations for 14 days: 0 mg/L (control), 10 mg/L (low), and 50 mg/L (high). Notably, low caffeine dose ameliorated the ability of fish to discriminate the cues and reach the target, spending most of the time close to the target where the reward was offered, and showing the shortest latency to reach the target. The higher dose impaired the ability to find the target, demonstrating increased anxiety, a possible side effect of the substance.

### 3.2.1.4. Selective attention

Higgins et al. [19] evaluated the effect of caffeine on Long-Evans (LE) and Cesarean-derived (CD) rats performance in a test of selective attention, the 5-CSRTT. The effects of caffeine were compared to the selective A<sub>2A</sub> antagonists, SCH 412348 and KW-6002, and the A<sub>1</sub> antagonist, DPCPX. Caffeine (3–10 mg/kg, i.p.) increased reaction time in both LE and CD rats, with no effect on accuracy, an effect replicated by SCH 412348 (0.1–1 mg/kg PO) and KW-6002 (1–3 mg/kg PO), but not DPCPX (3–30 mg/kg PO). The faster response speed was observed similarly in both the CD and LE rat strains at 3 mg/kg, although increased premature responses were confined to the LE strain at the 10 mg/kg dose. These results suggest that the attention-enhancing effects of caffeine were mediated through A<sub>2A</sub> receptor blockade. Selective A<sub>2A</sub> receptor antagonists may therefore have potential as therapies for attention-related disorders such as ADHD.

## 3.2.2. Hyperactivity and impulsivity

### 3.2.2.1. Locomotor activity

França et al. [20] tested the effect of caffeine on the hyperlocomotion characteristic of ADHD, by examining locomotor activity. Caffeine consumption (0.3 mg/mL in drinking water) plus physical exercise in running wheels during 6 weeks, either during adolescence (30 days old) or adulthood (4–5 months old), was not related to changes in spontaneous locomotor activity in SHR, in an open field, for 5 min, during the habituation phase of the object recognition task. Ruiz-Oliveira et al. [18] studied the effects of caffeine on zebrafish (4 months old, wild type, both sexes). Low concentrations of caffeine (10 mg/L) affected



locomotor parameters, increasing average speed and decreasing freezing behavior. Interestingly, the levels of freezing and locomotor behavior were the same for the 50-mg/L caffeine group and the control group. Nunes et al. [21] evaluated locomotor activity during the late childhood and the end of adolescence of male and female SHR, using an open field arena, and measuring the total distance travelled in meters along the periphery during 5 minutes. Although caffeine (0.3 g/L) did not impact hyperlocomotion during late childhood (PND 28) regarding both sexes, continuous treatment exacerbated the hyperactivity in adolescent female SHR (PND 50), suggesting that consumption of caffeine during childhood may exacerbate hyperactivity in females, only if the administration persists up to adolescence. Szczepanik et al. [22] demonstrated that young (3 months old) and middle-aged (8 months old) LDLr mice display different responses to chronic caffeine treatment, in terms of motor activity. Although caffeine was unable to modify the hyperlocomotion observed in 3 months old LDLr mice, caffeine attenuated the increased locomotor activity observed in 8 months old LDLr mice. Pandolfo et al. [15] tested if chronic treatment with caffeine was able to counteract hyperlocomotion characteristic of ADHD in SHR, during the open field test. Chronic treatment with caffeine did not alter central and total locomotion in SHR. Similarly, Pires et al. [23] showed that chronic treatment with caffeine did not produce changes in locomotor activity in SHR during the sample phase of the object recognition task. Interestingly, Caballero et al. [17] showed that neonatal 6-OHDA lesioned rats, another animal model of ADHD, performed a not significant tendency to decrease its motor activity after *ad libitum* caffeine consumption throughout the prepubertal period, during the Olton maze behavioral assay. Higgins et al. [19] conducted two separate types of locomotor activity studies. In a CGS-21680-induced hypolocomotion assay, pretreatment with caffeine (3–30 mg/kg, i.p.) produced a significant attenuation of the CGS-21680 hypolocomotion at doses of 10 and 30 mg/kg in CD rats. In a second experiment, caffeine (1–30 mg/kg, i.p.) produced a dose-related increase in locomotor activity in rats habituated to the test chambers. Finally, Prediger et al. [24] did not find any direct increase in locomotor performance in SHRs after administration of acute doses of caffeine (1–10 mg/kg i.p.), during the spatial version of the Morris water maze. No alteration was observed in the swimming speed, in this regard.

#### 3.2.2.2. Impulsive behavior

Leffa et al. [25] focused on impulsive behavior to elucidate the neurobiology of ADHD. They treated SHR with caffeine, a non-selective adenosine receptor antagonist, to evaluate the modulating effects of the adenosine systems in a tolerance to delay of reward task. Animals had to choose between a small, but immediate, or a large, but delayed, reward. An acute pretreatment with caffeine (2 mg/kg or 5 mg/kg) increased choices of large reward. Conversely, a chronic treatment with caffeine (2 mg/kg, for 21 days) increased the impulsive phenotype and decreased choices of large reward.

### 3.2.3 Learning and Memory

#### 3.2.3.1. Non-associative learning

Habituation is a form of non-associative learning in which the animal's innate response to a stimulus decreases after prolonged or repeated presentations of such stimulus.

Habituation, in animals, can be measured by a second exposure to the open field test, a form of non-associative learning. Nunes et al. [21] analyzed habituation during the late childhood and the end of adolescence of male and female SHR, by recording the total travelled distance during two consecutive days of exposure to the open field. These authors observed a sex and age difference in habituation, with female SHR showing lack of habituation since childhood, and male SHR showing lack of habituation in the adolescence. This lack of habituation observed in females, however, was reversed by caffeine (0.3 g/L) treatment during childhood.

#### 3.2.3.2. *Working memory*

The object-recognition task is recognized as a working memory task, relies on the animal's natural tendency for novelty, and tests the ability to discriminate between familiar and unfamiliar objects. França et al. [20] assessed working memory using an adapted version of the object recognition task, conducted in an open field during three different phases: habituation, sample and discrimination. Researchers measured the total time spent exploring during the sample phase. Although the results of this study showed that the disruption of the short-term recognition memory persisted into adulthood, the association of caffeine (0.3 mg/mL) and exercise during adulthood and adolescence improved short-term recognition memory in the SHR strain. Nunes et al. [21] carried out the novel object recognition test, as well, and observed similar recognition memory disturbances in adolescent SHR from both sexes. Nonetheless, caffeine intake (0.3 g/L) restricted to childhood restored recognition memory in adolescent SHR from both sexes. To evaluate the potential of caffeine in ADHD therapy, Pires et al. [23] treated female WKY rats and SHR with caffeine (3 mg/kg, i.p.) for 14 consecutive days during the prepubertal period. The animals were tested later in adulthood during the object-recognition task. While WKY rats discriminated all the used objects, SHR were unable to discriminate between pairs of objects with subtle structural differences. Nonetheless, chronic treatment with caffeine or MPD improved the object-recognition deficits in SHR. Pires et al. [26] showed, for the first time, significant impairment of SHR short-term object-recognition ability in comparison with WKY rats. They further investigated the effects of caffeine (1, 3 or 10 mg/kg), 30 min before the sample phase, on the performance of WKY rats and SHR of both sexes in the object-recognition task. Injection of caffeine (1, 3 or 10 mg/kg, i.p.) improved the discrimination index of female SHR, while the highest tested dose of caffeine (10 mg/kg, i.p.) increased the discrimination index of male SHR.

#### 3.2.3.3. *Spatial learning*

The water maze task is a behavioral procedure widely used with rodents to study spatial learning or spatial memory. Prediger et al. [24] used a circular swimming pool to assess the effect of caffeine administration on spatial learning deficit in SHR. Adult female WKY rats and SHR received caffeine (1–10 mg/kg i.p.) Thirty min before training, immediately after training, or 30 min before a test session. Pre-training administration of caffeine (1–10 mg/kg i.p.) improved spatial learning deficit in SHR. Post-training administration of caffeine (3 mg/kg i.p.) did not alter the SHR test performance, although increased memory retention in WKY rats. Although França et al. [20] observed impairments of procedural

memory in adolescent SHR during the cued version of the water maze, these normalized in adulthood.

#### 3.2.3.4 Spatial short-term memory

Given the willingness of rodents to explore new environments, the Y-Maze Test is widely used for testing the conditions affecting memory and learning. Pandolfo et al. [15] assessed spatial short-term memory in SHR, using a Y-maze paradigm. SHR displayed a deficit of spatial learning compared with WKY rats, the control group. Importantly, caffeine treatment (2 mg/kg, i.p.) during adolescence improved memory impairment exhibited by SHR. Nunes et al. [21] evaluated spatial memory in male and female SHR using the Y-maze task at PND 53. Female SHR showed worsened spatial memory. Although caffeine (0.3 g/L) was effective against recognition memory impairment in both sexes, only female SHR increased the percentage of entries in the novel arm after caffeine intake from PND 15 up to PND 55, and showed a spatial memory recovery.

#### 3.2.4. Olfactory discrimination

França et al. [20] evaluated the effects of caffeine consumption (0.3 mg/mL) plus physical exercise in running wheels during 6 weeks, either during adolescence (30 days old) or adulthood (4–5 months old), using SHR during the olfactory discrimination test. Besides providing the first evidence of deficits of olfactory discrimination in both adolescent and adult SHR, the authors showed how caffeine, together with physical exercise, was able to restore the olfactory discrimination ability in these animals during adolescence or adulthood.

#### 3.2.5. Blood pressure

França et al. [20] measured systolic blood pressure using the non-invasive tail-cuff method. For the animals treated during adolescence, the systolic arterial pressure was taken before (basal values) and 14, 28, and 42 days after beginning the treatment, before the behavioral tests. For the rats subjected to caffeine treatment and physical exercise during adult life, two measurements were taken, one before the protocols (basal values) and the other after the last behavioral task. Importantly, the hypertensive phenotype was not significantly altered by caffeine (0.3 mg/mL) or exercise. When applied from adolescence, caffeine and exercise had no effect on the development of hypertension and at 42 days of treatment (72 days of age) all SHRs were hypertensive. For adult animals that were already hypertensive at the beginning of the treatment, no further significant differences between groups were observed. To investigate if the SHR cognitive deficits could be directly associated with hypertension, Pires et al. [23] measured the effects of chronic administration of caffeine (3 mg/kg, i.p.) during the prepubertal period on the arterial blood pressure of adult female WKY rats and female SHR. As expected, SHR were hypertensive in comparison to WKY control rats. Chronic administration of caffeine during the prepubertal period, at the same doses that reversed the cognitive deficits of adult SHR (3 mg/kg, i.p.), did not cause significant changes in blood pressure values in adulthood SHR and WKY rats. Again, Pires et al. [26] measured blood pressure after caffeine treatment to investigate if the cognitive deficits of SHR could be directly associated with hypertension. To do so, arterial blood

pressure (mmHg) of female WKY rats and female SHR were measured 30 min after treatment with caffeine (1, 3, or 10 mg/kg, i.p.). As expected, SHR were hypertensive in comparison with WKY control rats. Nonetheless, the administration of the same doses of caffeine, which were able to improve the object-discrimination deficits of SHR, did not significantly alter the mean arterial pressure of either WKY rats or SHR. In a similar vein, Prediger et al. [24] measured the arterial blood pressure (mm Hg) of adult female WKY rats and SHR 30 min after injection of caffeine (1, 3 or 10 mg/kg, i.p.). Although SHR presented a significantly higher mean arterial pressure compared to WKY control rats, caffeine treatment did not significantly alter the mean arterial pressure of either WKY or SHR groups. Caffeine was therefore able to improve the spatial learning deficits in SHR without altering the hypertensive state, demonstrating that the cognitive impairment in SHR might not be entirely explained by hypertension.

### 3.2.6. Body weight

Pires et al. [23] measured the effects of chronic treatment with caffeine during the prepubertal period on the body weight of juvenile and adult female WKY rats and female SHR. The body weight of WKY rats and SHR was therefore measured every 2 days during the repeated treatment (14 days) with caffeine (1, 3, or 10 mg/kg, i.p.). The body weight of adult rats was also registered at the moment of the object-recognition task performance. Statistical comparisons indicated that juvenile rats from SHR strain presented significant lower mean body weight than juvenile WKY rats. Importantly, the chronic treatment with caffeine did not alter the body weight of the rat strains evaluated. During adulthood, similar results in the body weight of the animals were found. Although significant strain differences were observed, the chronic treatment with caffeine during the prepubertal period did not alter the final body weight of the animals in adulthood (regardless of strain). Similarly, Pandolfo et al. (2013) [15] found no weight differences between groups after treatment with caffeine (2 mg/kg, i.p.).

### 3.2.7. Neurobiology

#### *3.2.7.1. Brain levels of Synaptosomal-associated protein - 25*

França et al. [20] evaluated the effects of caffeine consumption (0.3 mg/mL in drinking water) plus physical exercise in running wheels during 6 weeks on the brain levels of monoamines, by high-performance liquid chromatography. Regarding SNAP-25 levels in the prefrontal cortex, statistical analysis revealed a significant increase in Synaptosomal-associated protein – 25 (SNAP – 25) levels in the prefrontal cortex in the group submitted to the association of caffeine consumption plus physical exercise. Regarding SNAP-25 levels in the hippocampus, statistical analysis indicated a significant increase of hippocampal SNAP-25 levels selectively in animals submitted to the association of caffeine consumption plus physical exercise.

#### *3.2.7.2. Brain levels of syntaxin*

SNAP-25 is a component of the soluble N-ethylmaleimidesensitive factor attachment protein receptor (SNARE) complex, critical to regulate synaptic vesicle fusion and

neurotransmitter release, along with syntaxin 1. Regarding Syntaxin levels in the prefrontal cortex, statistical analysis performed by França et al. (2020) [20] revealed a significant increase of syntaxin levels in the prefrontal cortex selectively in the group submitted to the association of caffeine consumption plus physical exercise. Regarding syntaxin levels in the hippocampus, statistical analysis indicated a main effect of treatment, with a marginal effect for treatment versus exercise interaction.

#### *3.2.7.3. Brain levels of serotonin*

França et al. [20] measured the effects of caffeine consumption plus physical exercise during adolescence on serotonin (5-hydroxytryptamine, 5-HT) by high-performance liquid chromatography (HPLC). Statistical analysis performed by the authors indicated that the association of caffeine consumption plus physical exercise during adolescence increased 5-HT levels in the prefrontal cortex of SHRs. Regarding hippocampal 5-HT levels, statistical comparisons indicated that caffeine consumption and physical exercise, alone or in combination, increased significantly hippocampal 5-HT levels.

#### *3.2.7.4. Brain levels of dopamine*

França et al. [20] also evaluated dopamine levels in the prefrontal cortex, hippocampus, and striatum by HPLC. Dopamine levels were not detectable in the hippocampus. Although a significant effect of treatment was observed in the prefrontal cortex, no significant effects were observed for exercise and their interaction. Statistical comparisons indicated no significant differences between groups in the levels of dopamine in the prefrontal cortex. Importantly, statistical analysis revealed significant effects of treatment, exercise, and their interaction in striatal dopamine levels. Subsequent statistical comparisons indicated that caffeine consumption and physical exercise, alone or in combination, increased significantly striatal dopamine levels.

#### *3.2.7.5. Dopamine transporter density*

Pandolfo et al. [15] investigated if cognitive and attention deficits of the SHR and their attenuation by caffeine treatment were associated with alterations of the density of Dopamine Transporter (DAT) in fronto-cortical and striatal terminals. The number of animals analyzed was 4 in the WKY control group, 4 in the WKY caffeine-treated group, 3 in the SHR control group and 4 in the SHR caffeine-treated group. Statistical analysis showed a significant effect of interaction between strain and treatment in the density of DAT in striatal and frontocortical synaptosomes. Therefore, DAT density was increased in both SHR brain areas of SHR and, importantly, caffeine treatment (2 mg/kg) during adolescence attenuated this enhanced DAT density in both brain areas of SHR, whereas caffeine treatment was devoid of effect in WKY rats.

#### *3.2.7.6. Dopamine uptake*

Pandolfo et al. [15] tested if this higher frontocortical density of DAT in SHR was accompanied by an increased uptake of dopamine. The authors directly measured dopamine uptake by synaptosomes. The number of animals was 4 per group. Both frontocorti-

cal and striatal synaptosomes from SHR took up almost the double amount of [ $^3\text{H}$ ]dopamine during the 3-min incubation period than the synaptosomes from WKY rats. Notably, chronic treatment with caffeine (2 mg/kg, i.p.) significantly reduced the dopamine uptake by synaptosomes from both brain areas from SHR when compared to vehicle-treated SHR, whereas caffeine was devoid of effects in WKY rats.

#### 3.2.7.7. Adenosine $A_{2A}$ receptor density

The effects of chronic caffeine consumption are generally attributed to the antagonism of  $A_{2A}\text{R}$ . Therefore, Pandolfo et al. [15] compared the density of  $A_{2A}\text{R}$  in striatal and frontocortical terminals from SHR or WKY rats treated with caffeine or saline. The number of animals analyzed was 4 in the WKY control group, 3 in the WKY caffeine-treated group, 4 in the SHR control group and 4 in the SHR caffeine-treated group. Statistical analysis indicated a significant effect of interaction between strain and treatment on  $A_{2A}\text{R}$  density both in the striatum and in the frontal cortex. Importantly, fronto-cortical nerve terminals from SHR displayed more colocalization between  $A_{2A}\text{R}$  and synaptophysin immunoreactivities than WKY rats. This provided the first direct demonstration of the presence of  $A_{2A}\text{R}$  in fronto-cortical nerve terminals, and the first indication that  $A_{2A}\text{R}$  density is enhanced in an animal model of ADHD.

#### 3.2.7.8. Colocalization of dopamine transporter and adenosine $A_{2A}$ receptors

Chronic caffeine treatment is proposed to operate through  $A_{2A}\text{R}$  and was shown to affect DAT density and function. Pandolfo et al. [15] finally proved the colocalization of  $A_{2A}\text{R}$  and DAT in striatal and frontocortical nerve terminals. The number of animals analyzed was 3 in the WKY control group, 4 in the WKY caffeine-treated group, 3 in the SHR control group and 3 in the SHR caffeine-treated group. While in the striatum, statistical analysis revealed a significant effect of strain on the colocalization of  $A_{2A}\text{R}$  and DAT immunoreactivities, and subsequent comparison showed that nerve terminals from vehicle-treated SHR displayed a significantly lower colocalization of  $A_{2A}\text{R}$  and DAT in comparison with vehicle-treated WKY, in the frontal cortex, statistical analysis revealed no significant effect of strain or treatment on the colocalization between  $A_{2A}\text{R}$  and DAT.

#### 3.2.7.9. Brain-derived neurotrophic factor

Nunes et al. [21] examined the effects of caffeine (0.3 g/L) administered since childhood in the brain-derived neurotrophic factor (BDNF) and its related proteins in both sexes of SHR, a rat model of ADHD. BDNF and its related proteins were therefore evaluated in the hippocampus of WKY and SHR from both sexes at PND 55. Statistical analysis revealed a significant effect of strain on BDNF levels, while the precursor form (proBDNF) remained unaltered. The TrkB receptor full length (TrkB-FL), phospho-TrkB, and truncated form of TrkB receptors were immunodetected in the hippocampus of WKY and SHR from both sexes. Statistical analysis revealed a significant effect of strain for the truncated form and also for phospho-TrkB. Moreover, the transcription factor CREB was not altered either by strain or sex, but its phosphorylated form (phospho-CREB) was increased in the hippocampus of SHR from both sexes. Finally, Nunes et al. [21] evaluated the impact of caffeine



only in the BDNF levels and TrkB receptors (TrkB-FL, phospho-TrkB, and TrkB-T). Caffeine administered from PND 15 up to PND 55 (caff/caff) decreased BDNF levels in the hippocampus from SHR male rats, while BDNF levels were unaltered in SHR female rats in both schedules of treatment. In male rats, caffeine in both schedules of treatment did not change either TrkB-FL or TrkB-T levels, while female SHR showed decreased TrkB-FL and TrkB-T forms by caffeine treatments. Both increased phospho-TrkB and CREB were not modified in the hippocampus from SHR after caffeine treatments.

#### 3.2.7.10. Neuronal development *in vitro*

Alves et al. [27] sought to investigate the effects of caffeine *in vitro*, at the neuronal level. At first, cultured frontal cortical neurons from SHR and WKY rats were immunostained for MAP-2 during their development *in vitro*. Later on, somatodendritic analysis were carried out, evaluating the number of branch points, roots, and maximal and total neurite length. Neurons from SHR, an animal model of ADHD, displayed less differentiation patterns, including neurite branching, shorter maximal neurite length and decreased axonal outgrowth. After 24 h of caffeine incubation (30  $\mu$ M), neurons from SHR presented a lower percentage of zero branch points, and a higher percentage of neurons with 2 branch points. A trend towards high percentage of neurons with 1 branch point was observed for SHR neurons after caffeine treatment. Caffeine also promoted an increase in the total and maximal neurite length in neurons from both strains. The authors further used either PKA or PI3K inhibitor in order to identify if one of the main transducing systems operated by adenosine receptors, and also in the neuronal differentiation, are involved in the effects observed by caffeine. The presence of PKA inhibitor KT5720 (5  $\mu$ M) did not change the ability of caffeine to enhance the percentage of SHR neurons with more branch points. The effect of caffeine in recovering the total neurite length of neurons from SHR was completely blocked by PKA inhibitor. Similar findings were found for maximal neurite length, in which PKA inhibitor completely attenuated the effects of caffeine. Finally, the authors also used LY294002 (50  $\mu$ M) as an inhibitor of PI3K and its presence blocked the effects of caffeine in increasing the number of branch points in neurons from ADHD model. Moreover, the effects of caffeine in preventing decreases in the total neurite length were abolished in the presence of PI3K inhibitor. The same findings were found for the maximal neurite length. The number of roots was also decreased by PI3K inhibitor in neurons from SHR.



Table 1. Summary of included studies.

Author/s & Year	Species, Strain, Sex & Sample (n)	Animal model	Age	Independent variables	Caffeine treatment	Behavioral tests / type of stress	Dependent variables	Main results
Szczepanik et al., 2016	Mice:  C57Bl/6 wild-type (8)  LDLr (8)  Female	Genetic (LDLr)	3-month old  8-month old	Treatment (caffeine or vehicle)  Strain (C57Bl/6 wild type or LDLr)	10mg/kg oral route  Chronic treatment (21 days)	Open-field arena	Spontaneous locomotor activity <i>(total distance travelled)</i>  Anxiety <i>(time in the center)</i>  Exploratory behavior <i>(visual inspection of the occupation plot)</i>	- LDLr mice travelled higher distances than the respec- tive C57Bl/6 wild type mice during the entire period (5 min) of analysis. - Caffeine treatment induced a renormalization effect in the locomotor activity of 8 month-old mice. - Caffeine treatment was unable to modify the hyperlo- comotion observed in 3 m.o.LDLr mice. - All animal groups spent a similar amount of time in the center of the open-field. - Similar exploratory behavior between groups.
Higgins et al., 2007	Rats:  LE (15-16)  CD (12-16)  Male	Not used	Not specified	Treatment (caffeine, SCH412348, KW-6002, DPCPX, CGS-21680, amphetamine)  Strain (LE or CD)	1 ml/kg i.p. route  One dose, prior testing	5-Choice serial reaction time task  Locomotor activity test	Selective attention <i>(Correct/incorrect trials, omis- sions, premature and persever- ative responses, choice accu- racy, correct/incorrect and magazine latency)</i>  Hypolocomotion <i>(distance travelled)</i>	- Caffeine, SCH 412348 and KW-6002 increased reaction time in LE and CD, with no effect on accuracy. - SCH 412348 effects were at doses not overtly psy- chostimulant. - CGS-21680 slowed reaction speed and increased omis- sions. A CGS-21680 low dose reduced the increased premature response caused by amphetamine. - Attention-enhancing effects of caffeine are mediated through A <sub>2A</sub> receptor blockade. Selective A <sub>2A</sub> receptor antagonists may have potential as therapies for atten- tion-related disorders.

Ruiz-Oliveira et al., 2019	Zebrafish  Wild-type (n=40)  Male  Female	Not used	4-month old	Treatment (caffeine or vehicle)   Chronic treatment (14 days)	- 10 mg/L - 50 mg/L drinking water   	Discrimination task	Conditioned learning ability <i>(average swimming speed, intergroup freezing, maximum speed, time spent in each area, latency to enter each area)</i>	- 0 and 10 mg/L caffeine groups spent most of the time close to the target.  - 10 mg caffeine group had the shortest latency to reach the target.  - 0 and 10 mg/L caffeine groups increased the average speed and distance travelled.  - Low-dose caffeine exposure seems to favor visual cue discrimination and to increase zebrafish performance in a multicue discrimination task.
Prediger et al., 2005	Rats  WKY (7-8) SHR (7-8)  Female	Genetic (SHR)	3-month-old	Treatment (caffeine or vehicle)  Strain (WKY or SHR)	- 1,3 mg/kg - 10 mg/kg i.p. route  One dose, prior testing	Water maze task	Spatial learning <i>(escape latency, distance travelled, swimming speed)</i>  Mean arterial pressure	- SHR needed more trials in the training session to acquire the spatial information, but a similar profile to that of WKY rats in the test session, demonstrating a selective deficit in spatial learning.  - Pre-training administration of caffeine improved spatial learning deficit in SHR.  - Post-training administration of caffeine did not alter the SHR test performance, but increased memory retention in WKY rats.  - No dose of caffeine altered mean blood pressure.
Pires et al., 2009	Rats  WKY (15) SHR (18)  Male Female	Genetic (SHR)	3-month old	Treatment (MPD, DPCPX caffeine, ZM241385 or vehicle)  Strain (WKY or SHR)	- 1 mg/kg - 3 mg/kg - 10 mg/kg i.p. route  One dose, prior testing	Object recognition task	Object recognition <i>(investigation time, discrimination time)</i>  Mean arterial pressure	- SHR only discriminated the most structurally distinct pairs of objects.  - Pre-training administration of MPD, caffeine, the selective adenosine receptor antagonists DPCPX and ZM241385, or the association of ineffective doses of DPCPX and ZM241385, improved the performance of SHR in the object-recognition task.

								- The administration of the same doses MPD and caffeine, did not significantly alter the mean arterial pressure of either WKY or SHR.
Pires et al., 2010	Rats  WKY (37) SHR (38)  Female	Genetic (SHR)	25/38 days-old	Treatment (caffeine, MPD or vehicle)  Strain (WKY or SHR)	- 3 mg/kg i.p. Route.  Chronic treatment (14 days)	Object recognition task	Object recognition ( <i>investigation time, discrimination time</i> )  Spontaneous locomotor activity  Mean arterial pressure  Body weight	- WKY rats discriminated all the objects. SHR were unable to discriminate pairs of objects with subtle structural differences.  - Chronic treatment with caffeine or MPD improved object-recognition deficits in SHR. These treatments impaired the short-term object-recognition ability in adult WKY rats.  - Drug effects were independent of changes in locomotor activity, arterial blood pressure and body weight.
Caballero et al., 2011	Rats  6-OHDA lesioned (9)  Saline-lesioned (9)  Male Female	Physical trauma (6-OHDA lesioned)	25-day-old	Treatment (caffeine or vehicle)	- 1 mg/ml drinking water  Chronic treatment (14 days)	Olton Maze behavioral assay	Motor behavior ( <i>number of arms crossed</i> )  Attention behavior ( <i>total number of arms walked, and total number of arms walked until one was repeated</i> )	- Caffeine treatment significantly improved the attention deficit of the 6-OHDA lesioned rats.  - No changes in the motor activity measurements were found before and after caffeine administration.

Pandolfo et al., 2013	Rats	Genetic (SHR)	24-day old	Treatment (caffeine or vehicle (saline))	- 2 mg/kg i.p. route	Attentional-set Shifting; Anxiety-related behavior; Ymaze; Locomotion related behavior	Attention <i>(regressive and never-reinforced errors, perseverative errors, total number of trials required before reaching 10 correct consecutive choices)</i>  Locomotion and Anxiety <i>(number of peripheral squares crossed, number of central squares crossed, percentage of central locomotion)</i>  Spatial recognition <i>(number of entries; time spent per arm; random exploration)</i>	- SHR were hyperactive and showed poorer performance in the attentional set-shifting and Y-maze paradigms, displayed increased dopamine transporter density and increased dopamine uptake in frontocortical and striatal terminals.  - Chronic caffeine treatment improved memory and attention deficits, and normalized dopaminergic function in SHR.  - First evidence of Adenosine A <sub>2A</sub> receptors (A <sub>2A</sub> R) in frontocortical nerve terminals  - First indication that A <sub>2A</sub> R density is enhanced in SHR.
Ouichi et al., 2013	Mice	Physical trauma (SI)	4-week old	Treatment (MPD and caffeine)	0.5-1 mg/kg i.p. route	Water finding test; Aggression; Modified Y-maze test; Novel object recognition test; Fear-conditioning test	Spatial attention <i>(entering &amp; drinking latency)</i>  Aggression <i>(duration of wrestling)</i>  Spatial recognition <i>(time spent in the new arm; total time exploring objects)</i>  Fear conditioning <i>(freezing behavior)</i>	- SI rats exhibited spatial attention deficit in the water-finding test. Re-socialized failed to attenuate the spatial attention deficit. The effect of SI on spatial attention showed no gender difference or correlation with increased aggressive behavior.  - SI significantly impaired contextual and conditional fear memory.  - MPD and caffeine improved SI-induced latent learning deficit in a manner reversible with cholinergic but not dopaminergic antagonists.

Nunes et al., 2018	Rats	Genetic (SHR)	15-days old	Treatment (caffeine/water, caffeine/caffeine or water)	0.3 g/L drinking water  Until PND 28	Open field test; Novel Object recognition;  Y maze task	Open field test  ( <i>travel distance periphery</i> )  Habituation  ( <i>total travelled distance in the open field</i> )  Spatial recognition- Y maze and object recognition  ( <i>exploration, discrimination ratio, number of entries, time spent in novel arm, total num- ber of entries in three arms</i> )	- Hyperlocomotion, recognition and spatial memory disturbances were observed in adolescent SHR from both sexes. Females showed lack of habituation and worsened spatial memory.  - Caffeine was effective against recognition memory im- pairment in both sexes. - Spatial memory was recov- ered only in female SHR.  - Female SHR showed exacerbated hyperlocomotion af- ter caffeine treatment.  - SHR from both sexes presented increases in the BDNF, truncated and phospho-TrkB receptors and also phospho-CREB levels in the hippocampus.  - Caffeine normalized BDNF in males and truncated TrkB receptor in both sexes.
Leffa et al., 2019	Rats	Genetic (SHR)	60/65- day-old	Treatment (WIN, AM251, caffeine or vehicle)	2 or 5 mg/kg i.p. route  Acute pretreatment, one dose  Strain (WKY or SHR)  Chronic treatment (21 days)	Tolerance to delay of reward;  T maze	Impulsive behavior  ( <i>tolerance to delay of reward</i> )	- WIN treatment decreased the choices of large reward and AM251 treatment with increased the choices of large reward in SHR  - Caffeine acute pretreatment blocked WIN effects.  - Caffeine chronic treatment increased the impulsive phenotype and potentiated the WIN effects.  - Cannabinoid and adenosine receptors modulate im- pulsive behavior in SHR.

Alves et al., 2020	Rats-pregnant  SHR (40-70)* WKY (40-70)* <i>*individual cells</i>  Female	Genetic (SHR)	In vitro	Treatment (caffeine, DMSO, LY294002, adenosine selective agonist and antagonists)  Strain (WKY or SHR)	Caffeine incuba- tion (30 µM)  One dose	No behavioral task	Morphological alterations <i>(singling, neurite branching)</i>	- SHR neurons displayed less neurite branching, shorter maximal neurite length and decreased axonal outgrowth.  - Caffeine recovered neurite branching and elongation from SHR neurons via PKA and PI3K signaling,  - A <sub>2A</sub> R agonist (CGS 21680) promoted more neurite branching via PKA signaling.  - The selective A <sub>2A</sub> R antagonist (SCH 58261) was efficient in recovering axonal outgrowth from SHR neurons through PI3K and not PKA signaling.
França et al., 2020	Rats  WKY (9)  SHR (11)  Male	Genetic (SHR)	30-day old  4-5 month old	Treatment (caffeine or water)  Physical exercise  Strain (WKY or SHR)	0.3 mg/mL, drinking water  One dose	Olfactory discrimination;  Open field;  Object recognition;  Water Maze	Olfactory discrimination <i>(time spent in compartments, numbers of crossings)</i>  Locomotor activity <i>(total distance, time spent in the central zone)</i>  Short-term memory <i>(total time spent exploring the objects, discrimination index)</i>  Working and procedural memories <i>(escape latency)</i>	- SHR displayed persistent olfactory and short-term recognition memory impairments from adolescence to adulthood, which were accompanied by lower levels of SNAP-25 in the prefrontal cortex and hippocampus.  - The association of caffeine plus physical exercise during adolescence or adulthood restored the olfactory discrimination ability and improved short-term recognition memory of SHRs.  - The association of caffeine consumption plus physical exercise during adolescence increased the levels of SNAP-25, syntaxin, and serotonin in the hippocampus and prefrontal cortex, and striatal dopamine levels in SHRs.

Key for abbreviations used: LDLr: low-density lipoprotein receptor, LE rat: Long-Evans rat, CD rat: Cesarean-derived rat, i.p.: intraperitoneally, WKY rat: wistar kyoto rat, SHR: spontaneously hypertensive rat, MPD: methylphenidate, 6-OHDA: 6-hydroxy-dopamine, A<sub>2A</sub>R: Adenosine A<sub>2A</sub> receptors, ICR mice: Institute of Cancer Research mice, SI: Social isolation, PND: postnatal day, BDNF: brain-derived neurotrophic factor, SNAP-25: synaptosomal-associated protein 25.

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#### 4. Discussion

ADHD is characterized by symptoms including attention deficits, impulsivity and hyperactivity that frequently persist throughout lifetime [1]. Modulation of prefrontal cortical function, and regulation of attention and behavior, relies on the optimum release of DA. Therefore, agents that can lead to increased release of DA or increased tonic firing of DA will be hypothetically beneficial in patients with ADHD by bringing prefrontal activity back to optimal levels [2,3]. In this sense, it has long been discussed whether caffeine could become an effective pharmacological compound for the treatment of ADHD symptoms.

This systematic review analyzed 13 animal studies that investigated the effects of caffeine in the modulation of ADHD-like symptoms. Taken together, the results indicate that caffeine treatment increases attention and improves learning, memory and olfactory discrimination without altering blood pressure and body weight.

Regarding attention, caffeine treatment improved attentional and behavioral flexibility on SHR [15], spatial attention on 6-OHDA lesioned rats [17] and SI on ICR mice [16], during adolescence. Caffeine treatment improved reaction time on LE and CD rats [19] and focus and attention on zebrafish [18], during adulthood.

Regarding learning and memory, caffeine treatment plus physical exercise during adulthood and adolescence improved working memory in SHR [20]. In the same vein, caffeine treatment alone restored non-associative learning in female SHR [21], improved working memory in SHR [23], female SHR [26] and adolescent SHR [21]. Administration of caffeine improved spatial learning deficit in SHR, increased memory retention in WKY rats [24] and improved spatial short-term memory in SHR [15] and female SHR [21].

Concerning olfactory discrimination, caffeine treatment, together with physical exercise, was able to restore olfactory discrimination in SHR during adolescence or adulthood [20]. Concerning blood pressure, caffeine treatment did not alter the hypertensive phenotype in SHR [24,26], during adolescence or adult life [20] and during the prepubertal period of adult female SHR [23]. Finally, caffeine treatment did not alter body weight in SHR [23,15].

If we are ever to acquire a truly in-depth understanding of the ADHD pharmacotherapy, we need to face the following question: *Does caffeine deserve a place in the arsenal of pharmacological agents for ADHD treatment, particularly during adolescence?* Although previous meta-analysis [28] and reviews [29] were unable to give any recommendation for adolescents diagnosed with ADHD, due to lack of data, our reviewed results provide updated preclinical evidence and support the therapeutic potential of caffeine to improve attention, learning, memory or olfactory discrimination in ADHD, especially during adolescence.

Beyond its clear effects on improving performance in tasks requiring attention, learning, memory and olfactory discrimination, without altering blood pressure and body weight, the implication of caffeine in modulating ADHD-like symptoms of hyperactivity remains controversial. Indeed, caffeine treatment plus physical exercise did not affect locomotor activity on SHR [20]. In like manner, caffeine treatment alone did not alter loco-



motion on SHR [24,23,15], preadolescent SHR [21] and young LDLr mice [22]. Nonetheless, caffeine treatment did increase locomotor activity on adolescent female SHR [21], zebrafish [18], produced a dose-related increase in locomotor activity in CD rats and a significant attenuation of a CGS-21680-induced hypolocomotion in CD rats [19], and attenuated locomotor activity on middle-aged LDLr mice [22] and 6-OHDA lesioned rats throughout the prepubertal period [17]. This apparent discrepancy could result from caffeine promoting different effects according to age and sex. In this regard, Nunes et al. [21] suggested that caffeine intake since childhood may exacerbate the hyperactivity in females, only if the administration persists up to adolescence. Szczepanik et al. [22] linked the age-dependent effect induced by caffeine with the idea that the blockade of adenosine A<sub>1</sub>/A<sub>2A</sub> receptors attempts to renormalize a potentially maladaptive system [30], being age an important escalating factor in mice. Otherwise, Ruiz-Oliveira et al. [18] proposed that caffeine-induced bursts of locomotion may be caused by a decrease in fatigue [31], rather than by an anxiogenic response. Importantly, the attenuation produced in motor activity by caffeine consumption, was pointed out as a natural effect of growth rather than an effect of caffeine intake by Caballero et al. [17].

As regard to impulsivity, although an acute pretreatment with caffeine increased choices of large reward on SHR, a chronic treatment with caffeine increased the impulsive phenotype and decreased choices of large reward on SHR [25]. This discrepancy may be explained by previous studies performed on animal models of brain diseases, showing that while an acute treatment acts mainly on A<sub>1</sub> receptors, a chronic treatment acts mainly on A<sub>2A</sub> receptors [32]. Leffa et al. [25], in this direction, underscored the ability of the adenosine modulation system to control behavioral inhibition.

Besides reviewing animal studies deciphering the effects of caffeine in the modulation of ADHD-like symptoms, we reviewed for the first time animal studies examining the effects of caffeine and adenosine receptors in neurons isolated from SHR, at the neuronal level.

In this respect, caffeine treatment plus physical exercise during adolescence increased the levels of SNAP-25, syntaxin, and serotonin in the hippocampus and prefrontal cortex, and striatal dopamine levels in SHRs [20]. In like manner, caffeine treatment alone during adolescence attenuated enhanced DAT density in fronto-cortical and striatal terminals of SHR and reduced the dopamine uptake by synaptosomes from SHR fronto-cortical and striatal terminals [15]. Furthermore, Pandolfo et al. [15] demonstrated that fronto-cortical nerve terminals are endowed with Adenosine A<sub>2A</sub> receptor, the target of chronic caffeine exposure, whose density was found to be increased in SHR. Caffeine treatment normalized BDNF levels in the hippocampus of SHR males, while the same treatment normalized TrkB receptors TrkB-FL and TrkB-T SHR in the hippocampus of SHR females [21]. Finally, neurons from SHR presented a lower percentage of zero branch points, and a higher percentage of neurons with 2 branch points, following an *in vitro* caffeine treatment consisting of 24 h of caffeine incubation. An increase in the total and maximal neurite length and a trend towards high percentage of neurons with 1 branch point was also observed for SHR neurons, after caffeine treatment. The effect of caffeine in increasing maximal neurite length, and in recovering the total neurite length of neurons from SHR, was

completely blocked by PKA inhibitor. LY294002, as an inhibitor of PI3K, blocked the effects of caffeine in increasing the number of branch points in SHR neurons. Finally, the effect of caffeine in preventing decreases in the total neurite length, increasing maximal neurite length and number of roots, was abolished in the presence of PI3K inhibitor in SHR neurons [27].

## 5. Conclusions

Overall, our reviewed data points out caffeine as a possible adjuvant pharmacological strategy for the treatment of ADHD. The compiled preclinical data supports the notion that caffeine improves ADHD-like symptoms of inattention, and its related learning and memory impairments, without affecting blood pressure and body weight. Our results are supported at the neuronal level, and strengthen the hypothesis that caffeine cognitive effects found in animal models could be translated to human ADHD, particularly during adolescence. Nonetheless, caution is needed when extrapolating animal studies potential effects in human patients. In this work, studies that explored caffeine effects on locomotor activity and impulsivity were contradictory, raising discrepancies that require further clarification. Although we consider that the results compiled in this systematic review can help to fill the gaps in scientific, pre-clinical and clinical knowledge regarding ADHD, future studies should be carried out not only to confirm and expand the available knowledge, but also to provide potential clues to support caffeine as a therapeutic approach for the treatment of ADHD.

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